INTRODUCTION

Insecticides are chemical substances that are used to eliminate and control insects. The majority of environmental pollutants like pyrethrins and pyrethroids are due to their popular usage as insecticides on farms for protecting crops and/or as weed killers or for domestic use in pest control (Dirinck et al. 2014). Pyrethrum has long been recognized as an extract having insecticidal properties and their active insecticidal compounds are called pyrethrins which the producers of flea powders were using as far back as 1800 in Asia. Synthetic pyrethroids are pesticides derived from naturally occurring pyrethrins which are gotten from pyrethrum (Thatheyus and Selvam, 2013). Some insects have however developed the ability to produce an enzyme that allows them to resist these pesticides. Most pyrethroids are now being produced with synergizing agents such as piperonyl butoxide which prevents the insects from breaking down the active ingredients; thus increasing the photostability and enhancing the insecticidal activity of these modified pyrethroids (Yu et al. 2010; Rattan et al. 2012).

Abstract

High malaria burden has led to an increased use of insecticides in the tropical and subtropical regions. Pyrethroids chemicals, commercially available pesticides, are greatly in use these days, thereby resulting in an elevated production of free radicals in subjects which can result in oxidative damage. The influence of pyrethroids based insecticides on peripheral and bone marrow cells was investigated using adult wistar rats. A total of 36 Wistar rats were randomly selected for the study and divided into two groups, twenty one rats were exposed to 1.2%w/v pyrethroids insecticides and the remaining rats grouped as non-exposed. Each group was further subdivided into three groups as 7-days, 21-days and 42-days of exposure groups respectively. Afterwards, the peripheral blood cells, bone marrow cells and the level of biomarkers of oxidative stress were assessed. Data were statistically analysed and level of significance was set at p<0.05. The mean red cell indices were significantly increased in the 42-days pyrethroids exposure than the 7-days exposure group. There was also an increase in the levels of expression of catalase (CAT) and hydrogen peroxide (H$_2$O$_2$) in the exposed groups while superoxide dismutase (SOD) showed significant reduction. Exposure to pyrethroids insecticides caused significant alterations in the haematopoetic elements and the severity of this pathological effect correlated with the duration of exposure. Pyrethroids insecticides can therefore cause oxidative stress and inflammation as well as peripheral and bone marrow perturbation in rats when exposed to as few as 7 days.

Key words: Pyrethroids, haematotoxicity, oxidative damage, free radicals
repetitive nerve impulses by delaying the closing of sodium ion channels and affecting the voltage-dependent inactivation of the sodium pump thereby causing a weakened state, paralysis and subsequently death (Soderlund et al. 2002). The use of synthetic pesticides in the developing countries has been increasing considerably owing to enhanced global food demands, vector-borne diseases, pests and their genetically modified resistant species (Sayim et al. 2012). Exposure to environmental factors such as pesticide is believed to adversely impact the function of biological systems vide the production of reactive oxygen species (ROS). These factors are important contributors of a wide range of diseases such as haematological dyscrasias, neurological, heart and reproductive diseases as well as cancer (Acquavella et al. 1996; Atere and Osadolor, 2017).

The bone marrow is found at the center of most bones and represents the major hematopoietic organ as well as the primary lymphoid tissue that is responsible for the production of erythrocytes, granulocytes, monocytes, lymphocytes and platelets. Pyrethrins and pyrethroids have been implicated as capable of causing cancers in humans, albeit the conclusion emanated from a study that fed animals large amounts of pyrethrins/pyrethroids for a lifetime (Lushchack, 2011). Other studies have also reported the hematotoxic potential of these pesticides (Kaufman, 1997; Assayed et al. 2010; Edem et al. 2012), there is however paucity of data on the effects of pyrethroids on erythroid precursors and developmental levels of blood components. The study is therefore designed to bridge this knowledge gap by evaluating the effect of oxidative damages on the peripheral and bone marrow indices in pyrethroids based insecticide exposed experimental model of toxicity in rats.

MATERIALS AND METHODS

Study Design:
This experimental study involved thirty six (36) inbred apparently healthy adult male Wistar rats weighing between 100 and 250g. The rats were obtained from the Department of Medical Biochemistry, Achievers University, Owo and housed within the facility and maintained on standard rodent pellets and water *ad libitum*. On transfer to the work area, the animals were allowed 14 days for acclimatization under standard conditions of temperature, relative humidity with 12-hour light and dark cycle. Animals were handled in line with good laboratory practice (GLP) and all experiments conducted in accordance with the National Institute of Health Guide for care and Use of Laboratory Animals.

Ethical Consideration:
Approval for this study was obtained from the biomedical research animal ethics committee of the Department of Medical Laboratory Science, Achievers University, Owo and Federal Medical Centre, Owo with registration number FMC/O/OW/380/VOL.LXII/97. The experiment was carried out in strict compliance with the standard guidelines for the care and use of animals for research in line with that set by the World Health Organization.

Selection of Insecticides:
Pyrethroids insecticides with the trade name “Raid” which is manufactured by SC Johnson and Son Nigeria Limited and registered with the National Administration of Food and Drug Agency Control (NAFDAC) was used for the experiment. This pyrethroids insecticide was selected for the study because of its registration with the National Administration of Food and Drug Agency Control (NAFDAC) for use in homes and as a multi-purpose insects killer containing both type I and II pyrethroids active ingredients like D-allethrin, tetramethrin and deltamethrin.

Experimental protocol
Rats were randomly grouped into two (2) which comprised of exposed and non-exposed groups. Each of the groups was further subdivided into three groups according to the duration of exposure to pyrethroid based insecticide with identification tag being given to rats in each group.

Group 1 (21 rats); TG,
Group 2 (15 rats); CG

Key: TG = treated group, CG=Control group.

All the experimental rats were housed in
small iron cages (36cm x 22cm x 14cm) with many holes and exposed to pyrethroid vapours inside a closed room (180cm x 240cm) according to the method described by Hasan et al. (2015). The animals were exposed to 1.2% w/v pyrethroid vapours for 8 hours daily for a period of 7-days, 21-days and 42-days respectively with the control group being kept under identical conditions without exposure to the pyrethroids chemicals. The description of the study was fully represented in figure 1.

Sample preparation

The rats in each group were sacrificed after 7 days, 21 days and 42 days exposure to the insecticide respectively under light ether anesthesia, blood specimens were collected from the inferior vena cava into ethylene diamine tetra-acetic acid (EDTA) bottle using 5ml syringe and processed immediately for complete blood count. Plasma was obtained from whole blood by centrifugation at 4000rpm for 5 minutes, into plain bottles and stored at -20°C until time of analysis for oxidative stress markers. Bone marrow smears were made after harvesting from the femur and stained using rowmannosky stain.

Analytical Methods

Haematological analyzer was employed for the determination of complete blood count (CBC). Bone marrow harvest, smear preparation, stain and microscopic view were done as described by Ordodi et al. (2006). Standard spectrophotometric technique was used in determining oxidative stress markers.

Statistical analysis

All the data were reported as Mean ± S.E.M. The results were statistically analyzed by one way analysis of variance (ANOVA) using statistical package for social scientist (SPSS) 23.0 version and Spearman's correlation used to test the association between the variables. P<0.05 was considered to be significant.

RESULTS

Table 1: Effects of exposure to pyrethroids on haematological parameters of rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>7-days exposure (n=7)</th>
<th>21-days exposure (n=7)</th>
<th>42-days exposure (n=7)</th>
<th>Non-exposed (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT (%)</td>
<td>43.86 ± 3.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.86 ± 6.77</td>
<td>49.71 ± 5.44</td>
<td>46.07 ± 5.44</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>14.53 ± 1.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.96 ± 2.27</td>
<td>16.69 ± 0.86</td>
<td>15.43 ± 1.85</td>
</tr>
<tr>
<td>RBC (X10&lt;sup&gt;12&lt;/sup&gt;/L)</td>
<td>4.86 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.99 ± 0.75</td>
<td>5.52 ± 0.29</td>
<td>5.13 ± 0.62</td>
</tr>
<tr>
<td>HCT: Haematocrit, HGB: Haemoglobin, RBC: Red blood cell, MCV: Mean cell Haemoglobin, MCH: Mean cell Haemoglobin, MCHC: Mean cell haemoglobin concentration. Values are expressed as mean ± SEM, *significantly different from 42 days exposure group (P&lt;0.05), †significantly different from 21 days exposure group (P&lt;0.05), ‡significantly different from non-exposure (control) (P&lt;0.05).</td>
<td></td>
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</table>

Table 2: Effects of exposure to pyrethroids on the expression of oxidative stress indices

<table>
<thead>
<tr>
<th>Parameters</th>
<th>7-days exposure (n=7)</th>
<th>21-days exposure (n=7)</th>
<th>42-days exposure (n=7)</th>
<th>Non-exposed (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/ml)</td>
<td>3.24 ± 0.81&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>1.99 ± 0.56</td>
<td>2.18 ± 0.51</td>
<td>2.27 ± 0.48</td>
</tr>
<tr>
<td>CAT (U/L)</td>
<td>33.84 ± 4.38&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>24.07 ± 2.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.71 ± 3.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.47 ± 3.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; (µmol/l)</td>
<td>3.69 ± 0.99&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>4.48 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.17 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.08 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
SOD: Superoxide dismutase, GPx: Glutathione peroxidase, CAT: Catalase, H$_2$O$_2$: Hydrogen peroxide; Values are expressed as mean ± SEM, 'Significantly different from non-exposure (control) at p<0.05, "Significantly different from 42-days exposure group," Significantly different from 21-days exposure group.

The mean red cell indices were significantly (p<0.05) increased in the 42-days exposure group compared with the other groups while the group with the 7-days insecticide exposure had the lowest level of MCHC. The plasma levels of oxidative stress markers were assessed in the exposed and non-exposed groups (Table 2). CAT and H$_2$O$_2$ levels were significantly (p<0.05) increased in the exposed groups than the non-exposed group. The group exposed to insecticide for 7 days showed a higher SOD levels than other groups.

Table 3: Correlation of oxidative stress indices with haematological variables in exposed groups

<table>
<thead>
<tr>
<th></th>
<th>SOD r-value</th>
<th>SOD p-value</th>
<th>CAT r-value</th>
<th>CAT p-value</th>
<th>H$_2$O$_2$ r-value</th>
<th>H$_2$O$_2$ p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT (%)</td>
<td>-0.121</td>
<td>0.601</td>
<td>-0.308</td>
<td>0.174</td>
<td>0.125</td>
<td>0.590</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>-0.190</td>
<td>0.410</td>
<td>-0.379</td>
<td>0.090</td>
<td>0.166</td>
<td>0.473</td>
</tr>
<tr>
<td>RBC (X10$^{12}$/L)</td>
<td>-0.131</td>
<td>0.571</td>
<td>-0.309</td>
<td>0.173</td>
<td>0.113</td>
<td>0.626</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>0.096</td>
<td>0.678</td>
<td>0.170</td>
<td>0.461</td>
<td>0.038</td>
<td>0.870</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>-0.158</td>
<td>0.493</td>
<td>-0.333</td>
<td>0.140</td>
<td>0.366</td>
<td>0.103</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>-0.373</td>
<td>0.096</td>
<td>-0.346</td>
<td>0.124</td>
<td>0.181</td>
<td>0.433</td>
</tr>
<tr>
<td>TWBC (X10$^9$/L)</td>
<td>0.547</td>
<td>0.010*</td>
<td>0.562</td>
<td>0.008*</td>
<td>-0.401</td>
<td>0.042*</td>
</tr>
</tbody>
</table>

Data presented as correlation coefficient (r), *Correlation is significant at the 0.05 level (2-tailed).

There was significant (p<0.05) positive correlation of SOD and CAT levels with TWBC there was however a negative correlation of H$_2$O$_2$ levels with TWBC when compared with the other groups exposed to insecticide (Table 3).
The percentage of exposed and non-exposed groups was 58.33% (21) and 41.67% (15) respectively as shown in the groups' distribution of all experimental animals (Figure 1).

**Photomicrographs of Bone Marrow Smear Among Exposed and Non-exposed groups**

The effects of pyrethroid insecticides exposure on haematopoietic elements were analysed in the bone marrow of the rats. There was a moderate increase in the myeloid erythroid ratio (M:E) amidst fatty cells of the bone marrow in the 7-days exposure group, haematopoietic elements were moderately proliferated amidst fatty cells with slight megakaryocytes increase in the 21-days exposure group. The haematopoietic elements were more proliferated with slight increase in megakaryocytes in the 42-days exposure group compared to the non-exposed group with normal M:E amidst fatty cells (Figure 3, 4, 5 and 2).

![Figure 2: Photomicrograph of bone marrow smear showing normal haematopoietic features in Control group (Leishman X40mag)](image1)

![Figure 3: Photomicrograph of bone marrow smear showing moderately increased M:E ratio amidst fatty marrow cells in the 7-days exposure group (Leishman x40mag)](image2)
DISCUSSION

Pesticides are chemical substances used for the elimination and control of insects, weeds and unwanted organisms and human contact with them may occur directly during fabrication, packaging and application or during consumption of contaminated foods (Gupta, 2006; Atere and Osadolor, 2017). Oxidative stress has been established to be harmful due to the production of oxygen free radicals which attack biological molecules such as lipids, proteins, and DNA (Ya-ting et al. 2014). The
Biomarkers of in-vivo oxidative stress have been attracting interest because the accurate measurement of such stress is necessary for the assessment of its role in lifestyle diseases as well as evaluating the efficacy of treatment. Recent studies have shown a correlation between free radicals and several disease states such as atherosclerosis, some cancers, cataract formation and other disorders involving the inflammatory response such as rheumatoid arthritis (Yoshikawa et al. 2002).

Blood represents an important index of the physiological and pathological status of the living body and deviations in these blood cell counts and depletion/elevation of plasma constituents outside established reference ranges often indicate haematoxicity (Dioka et al. 2002; Bin-Jaliah et al. 2014). The present study showed that the pyrethroids insecticide exposed rats developed anaemia, which usually is a manifestation of underlying disease process evidenced by the significant decrease in the levels of HCT, HGB, RBC, MCH and MCHC of the exposed groups with the lowest values observed in the 7-days group compared to the non-exposed group. These findings in the insecticide exposed groups suggest increased haemolysis and/or reduced erythropoiesis and the decrease in haemoglobin concentration could also have resulted from pyrethroids-induced impairment of haeme biosynthesis in the bone marrow suggesting a degree of anisocytosis (Bin-Jaliah et al. 2014). However, the 42-days exposure group showed a significant increase in the MCHC than the other exposed groups suggestive of macrocytic anaemia which could result from the physiological compensatory erythropoiesis of the rats geared towards overcoming the acute haemolytic condition earlier experienced. The haematological data of the present study are consistent with the findings of other previous studies where they reported aplastic anemia as being associated with pesticide exposure in farm workers and significant decreases in haematological parameters of rats exposed to 2, 2-Dichlorovinyl dimethyl phosphate chemical respectively (Kaufman, 1997; Edem et al. 2012).

Oxidative stress has been reported to result in increased free radical production associated with decreased antioxidants activity and it is noteworthy that pyrethroids-insecticide exposure of the rats also significantly reduced the plasma activity of SOD and CAT, but increased plasma levels of hydrogen peroxide in the days of exposure among exposed groups compared to the controls investigated in this study. This finding correlates with other studies on the effects of pesticides exposure on antioxidants activity (Surajudeen et al. 2014). This result established the presence of oxidative stress, which could be due to an increased production of free radicals or decreased activity of antioxidants as reported by Atere and Osadolor (2017). SOD plays a major role as the first line of antioxidant defense system by catalyzing the dismutation of superoxide radical to form hydrogen peroxide (an oxidant) and molecular oxygen (Edem et al. 2012).

Catalase catalyzes the decomposition of hydrogen peroxide to form water and oxygen; it is an essential enzyme in the protection of cells from oxidative damage by reactive oxygen species (ROS) (Igharo et al. 2016; Atere and Osadolor, 2017). It is therefore possible that an accumulation of \( \text{H}_2\text{O}_2 \) required to mop up these free radicals might account for this observation of reduced SOD and CAT activities. The observations of this finding suggest an increase in the formation of free radicals that could lead to oxidative damage due to the overwhelming antioxidant activities of these antioxidant enzymes.

The SOD and CAT positively correlated with the total white blood cell (TWBC) and an associated concomitant significant negative correlation of \( \text{H}_2\text{O}_2 \) with the TWBC in the pyrethroids exposed groups. Though not statistically significant, the observed leukocytosis in the 7-days exposed group could be reactive to inflammatory complications of pyrethroids exposure. The increase in the number of white blood cells in the peripheral blood of the pyrethroids treated rats is possibly due to these cells being an integral part of immunological responses against invading foreign antigens and modulation of allergic inflammatory response in line with earlier report by the International laboratory for research on animal diseases in 1990. This justifies the positive correlation of total white blood cells with enzymatic antioxidants and an inverse association with oxidative stress marker as reported in this study.
The bone marrow is the primary hematopoietic organ and lymphoid tissue that is responsible for the production of erythrocytes, leukocytes and platelets (Valli et al. 2002). Ingestion of a wide variety of plants and chemical substances has been reported to cause bone marrow infiltration and suppression of haematopoiesis which may result from impaired primary bone marrow dysfunction (Lund, 2006; Bin-Jaliah et al. 2014). In this study, pyrethroid insecticides exposure in rats was also accompanied with moderate increase in the M:E ratio amidst fatty cells of the marrow for the 7-days group which was evident of anaemia compared to the non-exposed group. Exposure to pyrethroids also caused moderate proliferations of the haematopoietic elements amidst fatty cells of the marrow in the 21-days and 42-days groups respectively. This could be ascribed to marked erythroid hyperplasia in response to acute anaemia observed in the pyrethroids exposed groups which is line with the report of Assayed et al. (2010) where they reported various alterations in the haematopoetic precursors of bone marrow as a strong indicator of the haematotoxicity of cypermethrin. The observed time-dependent moderate megakaryocytes in pyrethroids exposed groups could also be indicative of haemorrhagic complications of pyrethroids. This suggestion could additionally explain the observed polychromasia.

CONCLUSION

This study, therefore, shows that exposure to pyrethroid insecticides induced alterations in peripheral and bone marrow indices in rats with a time-dependent severity of the pathological effects of the exposure. The precise mechanism of the haematotoxicity of this pyrethroids-insecticide is however still not fully understood; hence, further investigation to study the antihuman globulin assessment, molecular mechanism of its toxicity and the reversibility of the pathological effects is recommended.

Conflicts of Interest: The authors declare that this manuscript was approved by all authors in its form and that no competing interest exists.

Funding: Self-sponsored

REFERENCES


