Haematological changes in rats exposed to insecticidal oils from the leaves of *Cassia occidentalis* and *Euphorbia milii*

Chibuzor Onyinye Okonkwo [a,*], Obioma Christopher Ohaeri [b], Item Justin Atangwho [a]

[a] Department of Biochemistry, University of Calabar, Cross Rivers State, Nigeria
[b] Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria

ABSTRACT

This study analysed the effect of insecticidal oils obtained from the leaves of *Cassia occidentalis* and *Euphorbia milii* on haematological indices in rats. It also evaluated the extent ethyl ether anaesthesia affects the concentration of haematological indices in experimental rats. Oils were extracted from both plants via soxhlet and administered to Wistar rats orally once a week, for 2 weeks. Fifty-six rats were divided into two groups (28 rats each), these were further divided into seven (7) groups of four rats each. The control group (A) received feed and water only, Groups B_1, B_2, and B_3 received 1500 mg/kg, 3000 mg/kg and 5000 mg/kg body weight of *C. occidentalis* oil extract, respectively, and similarly, groups C_1, C_2 and C_3 received 1500 mg/kg, 3000 mg/kg and 5000 mg/kg body weight of *E. milii* oil extracts. The first set was sacrificed under mild ethyl ether anaesthesia, while the second set was sacrificed without any anaesthetic agent. Thereafter, whole blood was drawn and analysed for changes in haematological indices. Both oils caused a significant ($P < 0.05$) decrease in white blood cells (WBC) and platelet counts at 5000 mg/kg body weight relative to the control in both unanaesthetised and anaesthetised rats. In addition, a significant decrease in MCH, MCHC and in Red Blood Cell counts and Packed Cell Volume was also observed in unanaesthetised rats exposed to *E. milii* oil and *C. occidentalis* oil respectively at 5000 mg/kg body weight relative to the control. Since insecticides are usually applied at much lower concentrations, the plant oils may be considered safe for use as insecticidal agents.

1. Introduction

Insects are chief pathogenic agents that cause many human, animal and plant diseases. They transmit pathogens that cause malaria, dengue fever, yellow fever and leishmaniasis resulting in low life expectancy throughout the world (Sachs and Malaney, 2002). Insects can cause economic damages and losses that can lead to starvation, particularly in underdeveloped countries like Nigeria. There is need for a credible alternative to the available pesticides particularly in the developing countries where there are inadequate occupational safety standards, protective clothing and washing facilities, insufficient enforcement, poor labelling, illiteracy and insufficient knowledge of pesticide hazards (Pimentel and Greiner, 1996).

Plant products have been in use as insecticides, insect repellents and insect anti-feedants for sometime now (Mordue, 1998). Many plant species contain substances that protect them from predators; these substances can be extracted and used to produce effective, natural insecticides. They are believed to possess many advantages over the synthetic pesticides (Muhammad, 2015). They manifest their insecticidal effects through toxicity, anti-feedant, growth inhibition, suppression of reproductive behaviour and reduction in egg production and fertility (Mostafa et al., 2012). In search of alternatives to synthetic pesticides, scientists have continued to study the efficacy of essential oils from aromatic plants. The discovery of bioactive secondary metabolites from plants which are toxic to herbivores that attack them opened the vista for their assessment as insecticides. These secondary compounds represent a large reservoir of chemical structures with biological activity (Duke et al., 2010).

The Government of Canada has initiated a program aimed at providing basic amenities for the development and implementation of lower-risk approaches in the management of pests (Agriculture and Agri-Food Canada, 2003). These sustainability programs continuously emphasize the importance of developing organic insecticides for pest control as it believed that natural insecticides present less risk to the environment than synthetic insecticides, in line with popular opinion of the public (James, 1990). The goal of an insecticide is to kill or reduce to

the barest minimum the damaging effects of insect pests and vectors of diseases. Essential oils are one of the most tested natural products against insect pests. They act by affecting some biological processes in the pest such as growth rate, life span and reproduction (Isikber et al., 2006). Influential scientific papers have also proposed a higher level of sustainability using natural products (Reganold et al., 2001) as the global use of synthetically produced pesticides have resulted in dare consequences. Among the biopesticides, botanicals are presently at the fore-front due to the eco-toxicological properties of the non-botanicals. Many secondary metabolites from various plants are popular for their insecticidal efficacies, and are sometimes used domestically to kill or minimize the impact of insect pests (Kim et al., 2010). Plants are very useful in ecological systems to control pests since they contain a rich source of bioactive chemicals (Pavela, 2008).

The importance of developing organic insecticides for pest control cannot be over emphasized as natural insecticides tend to present less risk to the environment than synthetic insecticides (Hammitt, 1990). Among the biopesticides, botanicals are presently at the fore-front due to the eco-toxicological properties of the non-botanicals (Okonkwo and Ohaeri, 2018a,b). Higher plants are rich sources of novel natural substances that can be used to develop environmentally safe products for insect control (Arnason et al., 1989). Oils extracted from the leaves of *E. milii* and *C. occidentalis* have been reported to possess reasonable levels of insecticidal efficacy against insect pests (Okonkwo and Ohaeri, 2018a). Panneerselvam and Murugan (2013) reported that *Cassia occidentalis* extract exerted moderate adulticidal effect against the adults of *Anopheles stephensis* with LC_{50} and LD_{90} values of 263.91ppm and 527.31ppm respectively. Kumar et al. (2014) also revealed that *C. occidentalis* exerted lethal action on the larvae of filarial vector. Kiran et al. (2015) reported that the leaf extract of *E. milii* exhibited certain levels of insecticidal action against Diamond back moth (*Plutella xylostella*). This research is aimed at assessing the possible toxicity of these oils on haematological indices of non-target species in the environment using albino rats as model.

The synthetic pesticides currently in use, such as the organophosphate and organochlorine insecticides have been associated with various forms of cancer, neurological disorders and lung irritations in humans (Mergel, 2010). Agriculturists who apply these insecticides in farms come in contact with these dangerous pesticides and may be prone to nervous system damages (Özkara et al., 2016). "Pesticide drift" may also occur as pesticides are sometimes carried by wind and water to non-target areas thereby penetrating groundwater, polluting streams and harming wildlife (Nash, 1994). There is therefore need for safer alternatives with less hazadous effects to man and the environment as a whole.

Cassia occidentalis also called coffee weed is a small tree that belongs to the Kingdom; *Plantae*, Division; *Rosopsida*, Order;*Fabales*, Family; *Fabaceae,*Sub-Family; *Caesalpiniaceae*, Genus;*Cassia,* Species;*Occidentalis* (Tomar, 2016). The specie gives off a foul odour when damaged. It is indigenous to Brazil and is found in warmer climates and tropical areas of South Central and North America (Okonkwo and Ohaeri, 2018a). In East Africa, it is commonly known as ant bush, arsenic bush or Negro coffee (De Philips and Krupnick, 2018). In Nigeria, it is known as Nigerian senna or stinking weed (Leslie, 2005), *Akidiogbara* by the Igbos, *Dora rai* by the Hausas and *Aboorere* by the Yorubas (Uzzi and Grillo, 2013).

Euphorbia milii known as crown of thorns, Christ plant or Christ thorn is a low-growing evergreen shrub with very thorny grooved stems and branches (Galasinao, 2015). It belongs to the family: *Euphorbiaciae*, phylum: *tracheophyta,* class: *magnoliopsida*, order: *euphorbiales*, genus: *euphorbia* specie: *milii* (IUCN, 2010). Legends associate it with the crown of thorns worn by Christ. It is not indigenous to Nigeria, but is believed to have been imported to Nigeria from India (Ombrello, 2015). A characteristic feature of all *Euphorbia* species including the crown of thorns, is the presence of milky latex which is secreted by the plant through broken stems, roots and leaves. The latex is usually poisonous and probably developed in order to protect the plant from herbivores (Ombrello, 2015).

The aim of this research is to ascertain the impact of oil extracts from the leaves of *C. occidentalis* and *E. milii* on some haematological indices in rats. The effect of ethyl ether anaesthesia on haematological indices of experimental rats relative to unanaesthetised rats will also be compared.

2. Materials and methods

Equipments used include:Soxhlet extractor Manufactured by B. BRAN Scientific and Instrument Company England, Thermo Scientific Rotary evaporator, Model R-300 USA, Electric blender Akai Tokyo Japan Model No: BDOO11DA-1033M made in PRC, Weighing balance Symmetry Colle-Parmer Instrument Co, USA, Sysmex KX-21N Haematology Analyser.

All chemicals used were of analytical grade and included; Diluent; cellpack (approximately 30ml was consumed per sample), WBC/HGB lyse reagent: Stromatolyser-WH (approx. 1.0ml per sample)(RBCs were lysed with the acid haemolytic reagent Stromatolyser-WH; this reagent selectively suppresses the degranulation of Basophils, resulting in their separation from other WBC), Detergent: Cellclean. All reagents were supplied ready for use and stored at the recommended storage conditions.

2.1. Collection, identification and extraction of oil from plants

E. milii and *C. occidentalis* plants were identified and deposited at the Herbarium Unit of the Department of Biological Sciences (Botany) University of Calabar Cross Rivers State Nigeria with Voucher numbers: Herb/Bot/Ucc/063 and Herb/Bot/Ucc/095A respectively. Leaves were thereafter harvested in the desired quantity from GPS mobile location Latitude; 4.961538, Longitude; 8.349273, No 4 Edim Otop close, off victory way, Satellite town Calabar, Cross Rivers State, Nigeria on the 18th of October 2015. The plants appeared healthy at the time of the harvest. The dried leaves of *E. milii* and *C. occidentalis* were pulverized into a fine powder using an electric blender. Oils were obtained by continuous extraction in Soxhlet apparatus for 16 h using n-hexane as solvent according to the method of Association of Official Analytical Chemists (AOAC, 1990).

2.2. Experimental animals

A total of fifty-six (56) rats were used. Rats were monitored from birth and separated from male rats at four (4) weeks; before attaining sexual maturity. They were adult female nulliparous and non-pregnant rats weighing between 130 and 150g, they were 8 weeks old at the time of this research. The study procedures were approved by the Faculty of Basic Medical Sciences (FBMS) University of Calabar-Animal Ethics Committee Ref no:FAREC/GP/005/16. The animal ethics procedures were complied with during the whole experimental process. Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments were examined and approved by the appropriate ethics committee. Rats were fed standard rat chow and tap water *ad libitum* with regulated temperature and humidity and a 12/12 h light-dark cycle. Rats were divided into two major groups; the first twenty-eight rats were sacrificed under light ether anaesthesia while the second group was sacrificed without any anaesthetic agent. Each group was further divided randomly into seven (7) groups containing four rats each. The control group (A) received rat feed and water only, Groups; B_1, B_2, and B_3 received 1500 mg/kg, 3000 mg/kg and 5000 mg/kg body weight of *C. occidentalis* oil extract, while groups C_1, C_2 and C_3 received 1500 mg/kg, 3000 mg/kg and 5000 mg/kg body weight of *E. milii* oil extracts. The administrations of oils were done orally once a week for 2 weeks. A preliminary estimation of LD_{50} of oils using Dixon's method (1965) revealed the LD_{50} to be higher than 5000 mg/kg. This was supported by studies conducted by Silva et al. (2011) (who reported that acute or subacute administration of *Cassia occidentalis* is not toxic in male and female Wistar rats, suggesting a

safety use by humans) and Ekram and Naija, (2006).

2.3. Blood sample collection and determination of haematological indices

At the end of 2 weeks, the first group were sacrificed under light ether anaesthesia, while the second group was fasted overnight, dazed and decapitated using a sharp sterile knife. Blood samples were drawn via cardiac puncture using a 2 ml sterile syringe. Exactly 1ml of blood sample for haematological analysis was dispensed into test tubes containing ethylene diamine tetraacetic acid (EDTA). This was used for Whole blood analysis (Full blood count) using Sysmex KX-21N Haematology Analyser following the manual of instruction. Red blood cells, haemoglobin concentration, haematocrit concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), White blood cell and platelet counts were determined (Sysmex KX-21N Operator's Manual October, 1999). This machine processes approximately 60 samples an hour and displays on the LCD screen the particle distribution curves of WBC, RBC and platelets along with data of 19 parameters. It also detects abnormal samples. The instrument employed three detector blocks and two kinds of reagents for blood analysis. The WBC count was measured by the WBC detector block using the DC detection method. This method detects the sizes of the blood cells by changes in direct-current resistance. Blood samples were aspirated, measured and sent to the applicable detector chamber. Inside the chamber, is an "aperture" containing electrodes on both sides. Between these electrodes flows the direct current. Blood cells suspended in the sample passed through the aperture, changing the direct-current resistance between the electrodes. The sizes of blood cells were detected via changes in the direct-current resistance, with such detections coming in the form of electrical pulses. The RBC count and platelets were measured by the RBC detector block using DC detection method. The HGB detector block measured haemoglobin concentration using the non-cyanide haemoglobin method.

2.4. Statistical analysis

Data was presented as mean ± standard error of mean (SEM). Statistical analysis was performed using one way analysis of variance (ANOVA) in the statistical package for social sciences (SPSS) for windows, version 20.0 (SPSS Inc., Chicago IL, USA).

3. Results

Both oils caused a significant ($P < 0.05$) decrease in white blood cells (WBC) and platelet counts at the highest dose of 5000 mg/kg body weight relative to the control in both unanaesthetised and anaesthetised rats. In addition to these, a significant decrease in MCH, MCHC and in Red Blood Cell (RBC) and Packed Cell Volume (PCV) was also observed in unanaesthetised rats exposed to *E. milii* oil and *C. occidentalis* oil respectively at 5000 mg/kg body weight relative to control.

4. Discussion

C. occidentalis oil caused a significant decrease in WBC, MCV and MCH in anaesthetised rats at 1500 mg/kg body weight relative to the control (Figs. 1 and 2); this was not the case in unanaesthetised rats where there was a significant decrease only in WBC at 1500 mg/kg body weight (Fig. 3). However, the decrease in MCV at 1500 mg/kg was also observed in anaesthetised rats that received *E. milii* oil (Fig. 6). This is suggestive of the fact that ethyl ether anaesthesia may affect iron synthesis and thus haemoglobin production in the blood. The decrease in MCV and MCH in anaesthetised rats administered *C. occidentalis* oil (Fig. 2), was also observed to be non-concentration dependent. Unlike *E. milii* oil, *C. occidentalis* oil caused significant decrease in WBC in unanaesthetised rats at all three concentrations and a decrease in PCV, RBC and platelet at 3000 mg/kg and 5000 mg/kg body weight relative to the control (Fig. 3), no significant change was observed in the derived parameters of this group (Fig. 4). This implies that *C. occidentalis* oil may cause anaemia and reduce immunity to diseases and infections at very high concentrations. Nabukenya et al. (2014) reported that 600 mg/kg dose of aqueous extracts of *C. occidentalis* exerted significant decrease in AST, ALT but increased urea and uric acid relative to the control, they concluded that *C. occidentalis* was hepato-protective. However, according to Panigrahi et al., (2014a), *Cassia occidentalis* seed consumption is the main etiological factor in children population suffering from hepatomyoencephalopathy in India. They also predicted the involvement of multiple pathways and related biomolecules in CO induced hepato-toxicity; they claimed that their data may be useful in formulating strategies for therapeutic interventions of suspected CO poisoning study cases. In 2015, Panigrahi et al. also reported that AQ aglycones are responsible for producing toxicity, which may be associated with symptoms of hepatomyoencephalopathy in CO poisoning cases. Also, according to Vashishtha et al. (2009), the clinical spectrum and histopathology of *C. occidentalis* poisoning in children resemble those of animal toxicity, affecting mainly hepatic, skeletal muscle and brain tissues. In this study however, most changes in haematological indices occured at very high concentrations (3000 mg/kg and 5000 mg/kg body weight) as compared to lower concentrations usually required for insecticidal action. Various studies on the insecticidal effects of *C. occcidentalis* have
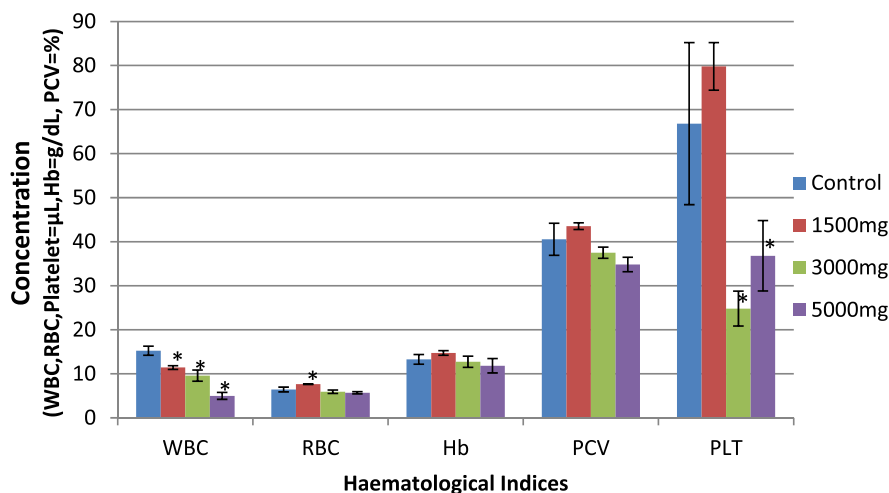

Fig. 1. Effect of *C. occidentalis* oil on haematological indices of anaesthetised rats. WBC – White blood cell, RBC – Red blood cell, Hb – Haemoglobin, PCV – Packed cell volume, PLT – Platelet.
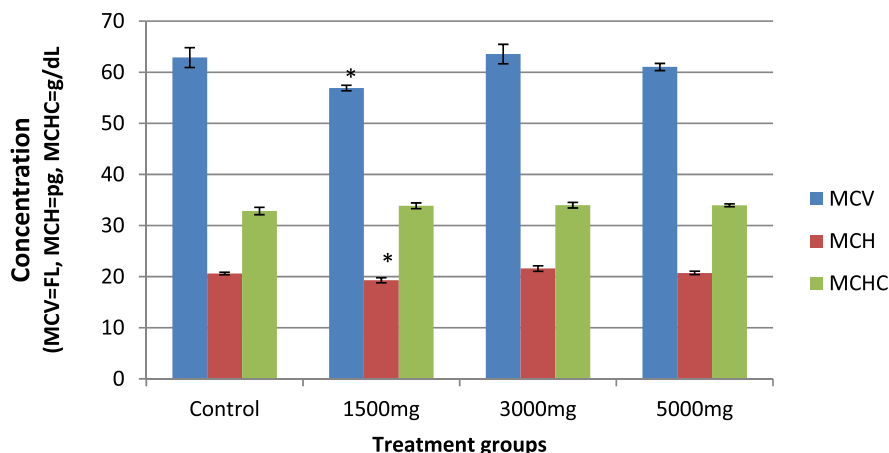
Fig. 2. Effect of *C. occidentalis* oil on derived indices of anaesthetised rats. MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular haemoglobin Concentration.
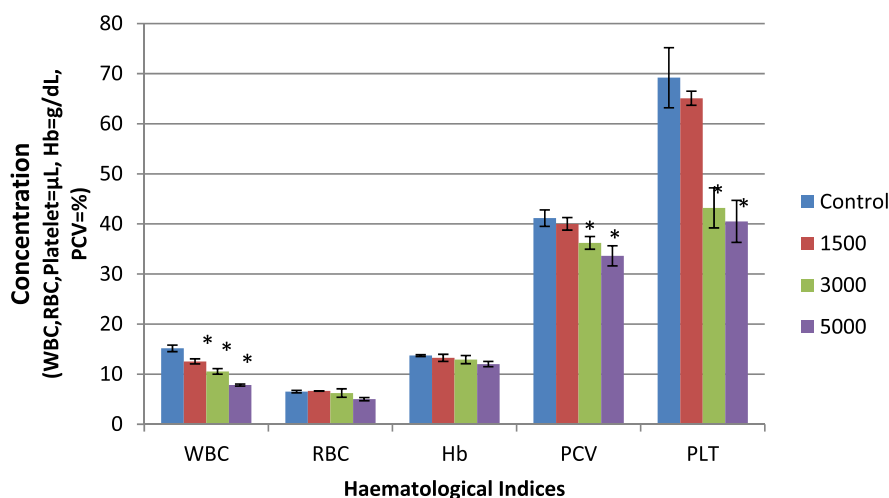

Fig. 3. Effect of *C. occidentalis* oil on haematological indices of unanaesthetised rats. WBC – White blood cell, RBC – Red blood cell, Hb – Haemoglobin, PCV – Packed cell volume, PLT – Platelet.
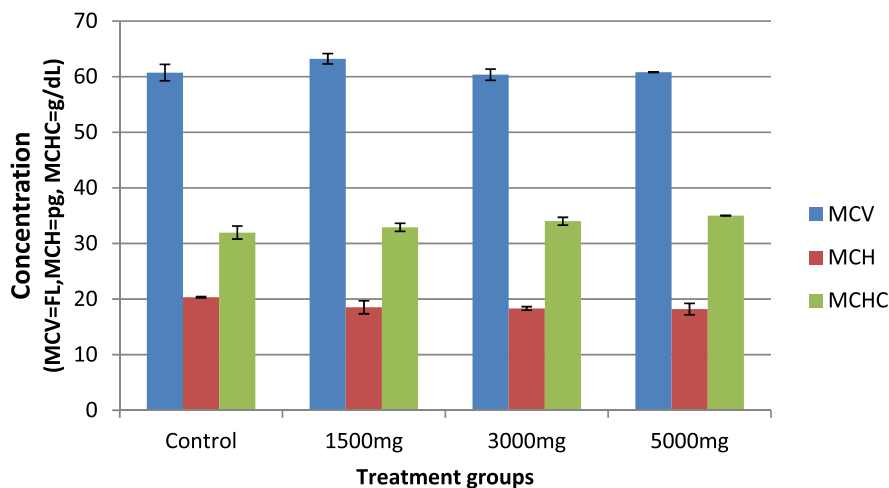

Fig. 4. Effect of *C. occidentalis* oil on derived indices of unanaesthetised rats. MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular haemoglobin Concentration.

shown that the plant extract usually exert insecticidal action from the doses of 43.7 ppm (0.437 g in 1000 ml) to 1000 ppm (one gram in 1000 ml). These doses may vary depending on environmental factors and varying plant species which may not be the same world wide. Kumar
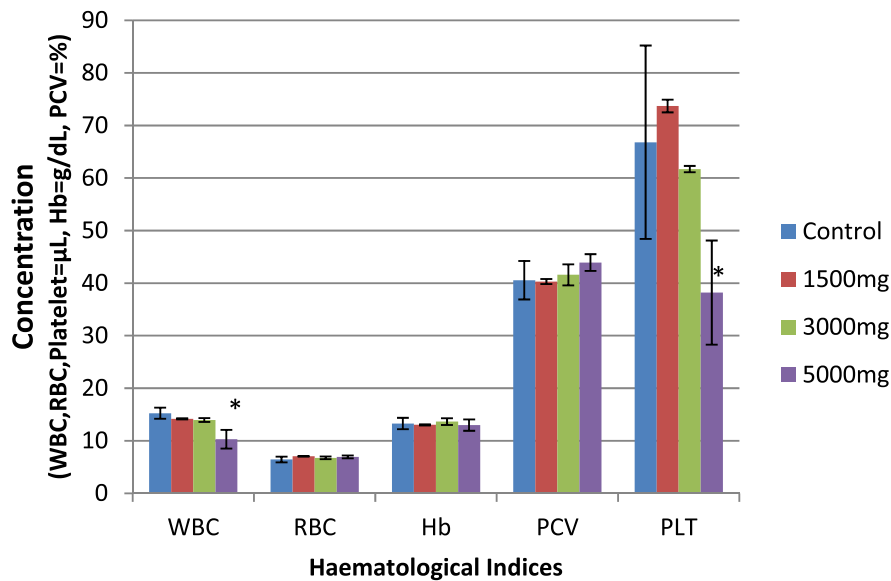
Fig. 5. Effect of *E. milii* oil on haematological indices of anaesthetised rats. WBC – White blood cell, RBC – Red blood cell, Hb – Haemoglobin, PCV – Packed cell volume, PLT – Platelet.
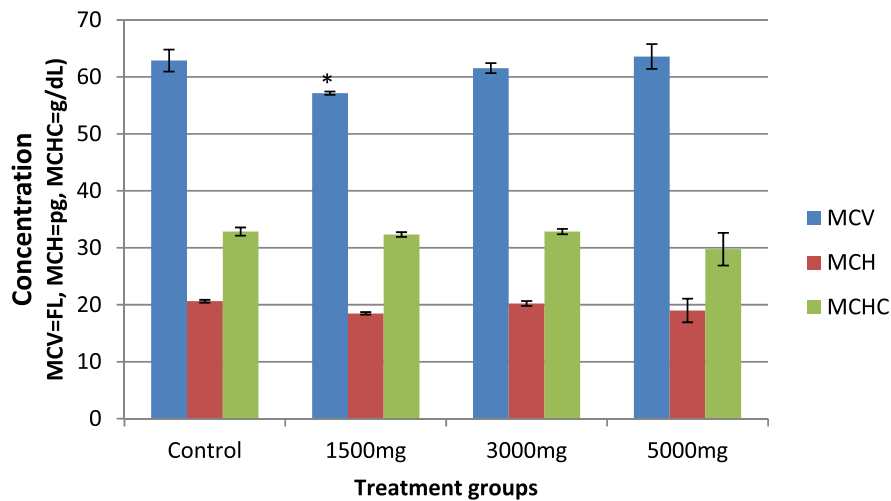

Fig. 6. Effect of *E. milii* oil on derived indices of anaesthetised rats. MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular haemoglobin Concentration.

et al. in 2012 reported that *C. occidentalis* leaf extract had LC_{50} and LC_{90} values of 74.67 and 202.35 ppm respectively against fourth instars of dengue vector and resulted in 100% mortality at 1000 ppm for 24 h. Panneerselvam and Murugan, (2013) also reported 100% mortalty of the malaria vector; *Anopheles stephensis* when exposed to the methanol leaf extract of *C. occidentalis* at 300ppm. They also reported LC_{50} and LC_{90} values of 263.91 and 527.2ppm respectively for *C. occidentalis* extract. Kumar et al. (2014) in their study of the larvicidal activity of *C. occidentalis* against the larvae of *Culex quinquefasciatus* also reported 100% mortality effect of petroleum ether and butanol extract of *C. occidentalis* at 200 and 300ppm respectively. Okonkwo and Ohaeri in 2018a, reported the insecticidal toxicity of *E. milii* and *C. occidentalis* plant extracts at 500ppm (500 mg/kg) body weight on *Periplaneta americana, Tettigonia viridissima* and *Anopheles gambiae*. Kiran et al. in 2015 also reported the larvicidal action of *E. milii* plant extract against 2nd instar larvae of field collected *P. xylostella* with LC_{50} value of 43.7ppm. From these studies it is clear that the concentration required for insecticidal action of these plant extracts is much lower than the doses used in this animal study.

Though *C. occidentalis* caused significant decrease in WBC, MCV and MCH at 1500 mg/kg body weight while *E. milii* caused significant decrease in MCV at the same concentration, it is reasonable to consider them safer than the synthetic pesticides whose toxicities are manifested at much lower doses. For instance, Deltamethrin, a synthetic pyrethroid insecticide have been reported to cause increase in salivation, lack of coordination, muscle tremor and convulsions with LD_{50} value of 150 mg/kg in rats (Manna et al., 2005). Hussain et al. (2009) have also observed significantly lower body weight gain in cypermethrin-treated rats at 500 mg/kg body weight relative to the control. According to Grewal et al. (2010), cypermethrin produced mild to moderate toxicosis characterized by intermittent diarrhoea, decreased feed intake, thick eye discharge and some degree of mortality at a dose of 5 mg/kg. Also, while the LD_{50} of SWAN insecticide is 4000 mg/kg as indicated by its manufacturers, the LD_{50} of both plant extracts has been reported to be above 5000 mg/kg (Silva et al., 2011) (Ekram and Najia, 2006). This shows that the extracts may be safer to animals than most synthetic insecticides.

Silva et al. (2011) reported that subacute treatment with *C. occidentalis* (100mg, 500mg and 2500 mg/kg) did not change body
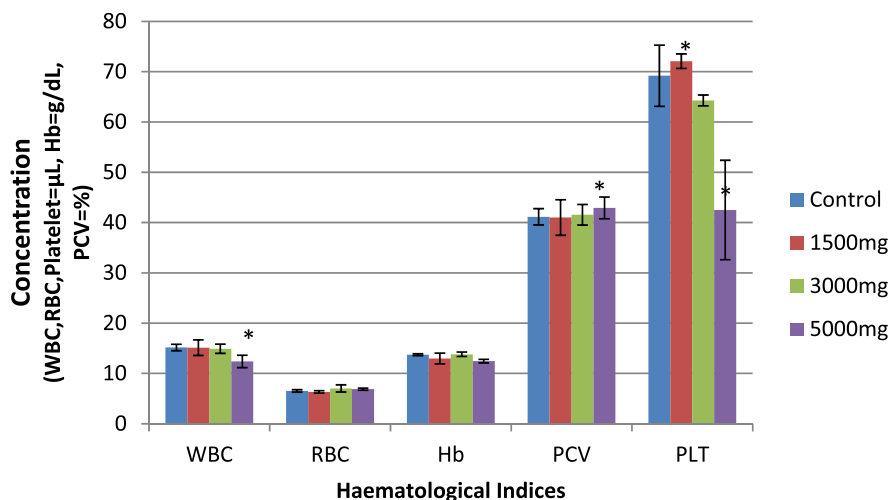
Fig. 7. Effect of *E. milii* oil on haematological indices of unanaesthetised rats. WBC – White blood cell, RBC – Red blood cell, Hb – Haemoglobin, PCV – Packed cell volume, PLT – Platelet.
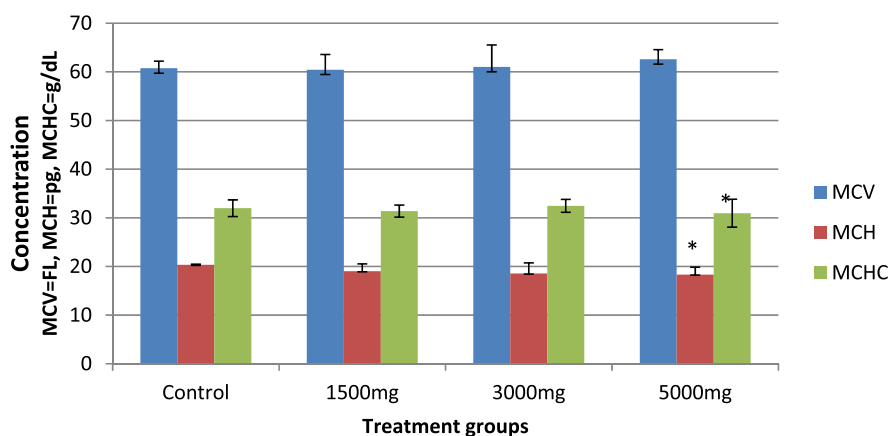

Fig. 8. Effect of *E. milii* oil on derived indices of unanaesthetised rats. MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular haemoglobin Concentration.

weight gain, food and water consumption, haematological and bochemical profiles in rats, they also reported LD_{50} of greater than 5000 mg/kg body weight for *C. occidentalis*. They observed no change in macroscopic and microscopic aspects of organs in animals administered *C. occidentalis*. From their research, acute and subacute administration of *C. occidentalis* is not toxic in male and female wistar rats, suggesting a safety use by humans. This does not totally agree with our findings from this research given that significant changes in some haematological indices were observed with *C. occidentalis* even at 1500 mg/kg body weight, however these differences may be as a result of varying sspecies and environmental differences at the study locations. Nuhu and Aliyu (2008) in another study titled "the effects of Cassia *occidentalis* aqeous extract on biochemical markers of tissue damage in rats, observed hypoproteinamic effects and increase in ALT, ALP and AST of rats treated with *C. occidentalis*. According to them, the crude extracts of *C. occidentalis* leaves may be slightly toxic as concotion for liver ailments, this agrees with the current study.

Considering the importance of the white blood cells in maintaining the integrity of the immune system, its reduction in the blood may cause a compromise in the immune defense system of mammals. A decrease in the number of red cells in the blood as observed with *C. occidentalis* at 5000 mg/kg body weight in unanaesthetised rats (Fig. 3) is often associated with the development of anaemia (Junqueira and Carneiro, 2006). Red blood cells (RBC) or erythrocytes, are the most common type of

blood cells and the vertebrate organism's principal means of delivering oxygen (O_2) to the body tissues through the circulatory system (Eric, 1995). This could be due to the stimulation of lipid peroxidative system by toxins resulting in the production of lipid peroxides which haemolyse the red blood cells (Kolanjiappan et al., 2002). The decrease in haematocrit (PCV) of rats observed at 5000 mg/kg body weight of *C. occidentalis* in unanaesthetised rats suggests that at very high concentrations (5000 mg/kg body weight) *C. occidentalis* oil may induce anaemia and inability of the cells to deliver oxygen to body tissues that require them. Packed Cell Volume (PCV) or Haematocrit is used clinically to signal known or suspected anaemia (Wintrobe and Greener, 2009). Since the purpose of red blood cells is to transfer oxygen from the lungs to body tissues; a blood sample's haematocrit can become a point of reference of its ability to deliver oxygen (Wintrobe and Greener, 2009). Factors influencing RBCs will affect the haematocrit because RBCs comprise 99% of the total cells of the blood (Wintrobe and Greener, 2009). A lower than normal haematocrit is representative of anaemia especially the aplastic anaemia and some thalassemia syndromes (Tamariz et al., 2008).

The significant ($P < 0.05$) decrease in white blood cells (WBC) relative to the control group at 5000 mg/kg body weightin anaesthetised rats administered both oils may indicate that the oils at very high concentrations may induce bone marrow deficiency or failure (Vajpayee et al., 2011). Low white blood cell count (Leukopenia) is usually caused by bone marrow problems especially when exposed to certain chemicals like

benzene and pesticides which can hurt the bone marrow's ability to make leukocytes (Burgess, 2017). This increases the risk of infection since leukocytes help to fight off infection and they are a vital part of the immune system (Burgess, 2017).

The decrease in platelet concentration in this group (Fig. 3) shows that the oils may affect the production and or cause the destruction of platelets at very high concentrations (5000 mg/kg body weight). Low platelet count is usually associated with the use of certain drugs which confuse the immune system and cause it to destroy platelets (Walker, 2016). Examples include heparin, quinine, sulfa-containing antibiotics and anticonvulsants (The Merck Manual, 2015). The non significant ($P >$ 0.05) change in red blood cell count (RBC), haemoglobin concentration (HB), packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in anaesthetised rats administered *E. milii* oil relative to the control (Figs. 5 and 6) suggests that the oil extract may be safe for use as insecticide to a very large extent. This agrees with the report of Souza et al. (1997) which revealed that *E. milii* latex posed no teratogenic hazards or toxic effects on rats. From their report, the possibility of toxicity or teratogenicity is of a considerably low order of magnitude.

Apart from the decrease in WBC and platelet (Fig. 7) in unanaesthetised rats administered *E. milii* oil, there was also significant ($P <$ 0.05) decrease in MCH and MCHC (Fig. 8) at 5000 mg/kg body weight relative to the control. Since this decrease was not observed in anaesthetised rats given *E. milii* oil, it is possible that the use of ethyl ether anaesthesia has buffered or masked the actual effect of this oil extract on MCH and MCHC. The mean corpuscular haemoglobin is a measure of the concentration of haemoglobin in a given volume of packed red blood cells (Todd, 2014). It is reported as part of a standard complete blood count; calculated by dividing the haemoglobin concentration by the haematocrit. It is mainly used in the diagnosis of iron deficiency (Todd, 2014). A low MCHC is a sensitive indicator of iron deficiency. Decreased levels of MCHC values as observed in this group may indicate abnormal haemoglobin synthesis, failure of blood osmoregulation and plasma osmolarity (Stookey et al., 2007). This means that very high concentrations of *E. milii* oil may induce iron deficiency anaemia in experimental rats.

5. Conclusion

The plant oil extracts may be considered practically safe as insecticidal agents since their toxic effects are manifested only at very high concentrations (3000 mg/kg and 5000 mg/kg body weight) as compared to the minute concentrations usually required for insecticidal action. The results also indicate that the use of ethyl ether anaesthesia in sacrificing experimental animals may significantly ($P < 0.05$) affects results obtained from such experiments. Thus, the use ethyl ether anaesthesiain the sacrifice of experimental animals may be reviewed as it obviously affects some haematological indices.

Declarations

Author contribution statement

Chibuzor Onyinye Okonkwo: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Obioma Christopher Ohaer: Conceived and designed the experiments; Analyzed and interpreted the data.

Item Justin Atangwho: Contributed reagents, materials, analysis tools or data, Wrote the paper.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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