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Research Article
Safety of Insecticidal Oils from *Cassia occidentalis* and *Euphorbia milii* on Biochemical Markers of Tissue Integrity

[1]Okonkwo Chibuzor Onyinye, [2]Ohaeri Obioma Christopher and [2]Atangwho Justin Item

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Cross Rivers State, Nigeria
[2]Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Abstract
Background and Objective: A major concern in the development of insecticides is their safety to non-target species. This study aimed to analyze the safety of botanical oils with insecticidal properties on albino rats. **Materials and Methods:** Twenty-eight rats were divided into 7 groups of 4 rats each. The control group (A) received feed and water only, Groups: B_1, B_2 and B_3 received 1500, 3000 and 5000 mg kg^{-1} b.wt., of *C. occidentalis* oil extract, while groups C_1, C_2 and C_3 received 1500, 3000 and 5000 mg kg^{-1} b.wt., of *E. milii* oil extracts. Oils were extracted from both plants via soxhlet extraction; extracted oils were administered to albino rats orally once a week for 2 weeks. Thereafter, animals were sacrificed and serum analyzed for kidney and liver function markers. **Results:** Both oils caused a significant ($p<0.05$) increase in AST and ALP activities and decrease ($p<0.05$) in ALT activity, creatinine and urea concentrations at 3000 mg and 5000 mg kg^{-1} b.wt., relative to the control. *Cassia occidentalis* oil also caused significant ($p<0.05$) decrease in albumin concentration at 5000 mg kg^{-1} b.wt. **Conclusion:** The oils may be considered safe for insecticidal applications, since lower concentrations are required for insecticidal action.

Key words: Insecticidal, *Cassia occidentalis*, *Euphorbia milii*, tissue integrity, botanical, biochemical markers

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Corresponding Author: Okonkwo Chibuzor Onyinye, Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar Cross Rivers State, Nigeria Tel: +2348068758063

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Insects are major enemies of human, livestock and agricultural crops all over the world. They transmit pathogens that cause malaria, dengue fever, yellow fever and leishmaniasis resulting in low life expectancy throughout the world. Nearly half of the world's population is infected with at least one type of insect-borne pathogen or the other. Malaria alone affects up to 300 million persons yearly and thrives more in areas of poverty and low economic growth[1]. Insecticides are important tools used for the control of insect pests and reduction in the damages they cause, in order to increase agricultural crop yield and improve the quality of life for humans, domestic animals and livestock. The sustainability of insect pest control is a very interesting topic currently receiving attention in the agric and agric-related literature[2]. A study in 2001 has favoured an organic management approach[3]. Several governments have taken initiatives to develop environmentally friendly agricultural practices[4]. These sustainability programs continuously emphasize the importance of developing organic insecticides for pest control as they assumed that natural insecticides present less risk to the environment than synthetic insecticides, which agrees with the general opinion of the public[5].

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[6]. Agriculturists who apply these insecticides in farms come in contact with these dangerous pesticides and may be prone to nervous system damages. "Pesticide drift" may also occur as pesticides are sometimes carried by wind and water to non-target areas. They penetrate groundwater, pollute streams and harm wildlife[7]. Cypermethrin; one of the active ingredients in most synthetic insecticides has been reported to cause toxicity to the reproductive system in male rats. After 15 days of continual dosing, both androgen receptor levels and serum testosterone levels reduced significantly[8]. Long-term exposure in adulthood is found to induce dopaminergic neuro-degeneration in rats and postnatal exposure enhances the susceptibility of animals to dopaminergic neuro-degeneration if rechallenged during adulthood[9]. Genetic damage, chromosomal abnormalities, increases in bone marrow and spleen cells are some of the diseases associated with exposure to cypermethrin[10]. Cypermethrin has also been implicated as a possible human carcinogen and is linked to an increase in bone marrow micronuclei in both mice and humans[11]. Studies have also shown that residue from cypermethrin can last for 84 days in the air, on walls, the floor and on furniture[12] relative to natural pesticides which are degraded shortly after application. Again, being a broad-spectrum insecticide, it kills both beneficial insects and animals together with the target pests[13]. It is believed to affect the insect's nervous system and is found in many household insecticide products. Contact with the skin may cause irritation, while inhalation may cause shortness of breath. It has a low solubility in water, making it unlikely to contaminate water sources in run off[14].

There is thus need for a credible alternative to the available synthetic 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[15]. Higher plants are rich sources of novel natural substances that can be used to develop environmentally safe products for insect control[16]. 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[17]. The discovery of bioactive secondary metabolites from plants which are toxins 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[18]. Essential oils from the leaves of *E. milii* and *C. occidentalis* have been reported to possess reasonable levels of insecticidal efficacy against insect pests[19]. Venkatesan *et al.*[20] also reported that crude extract from *Cassia occidentalis* showed potential growth regulation and a moderate level of adulticidal activity against the urban malaria vector; *Anopheles stephensis*. The safety of these oils towards non-target species have however not been reported.

Euphorbia milii also known as crown of thorns, Christ plant or Christ thorn is a low-growing evergreen shrub with very thorny grooved stems and branches. It belongs to the family: Euphorbiaceae, phylum: tracheophyta, class: magnoliopsida, order: euphorbiales, genus: *Euphorbia* specie: *milii*[21]. 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[22]. It is currently found all over the world as widely grown ornamental specie[23]. 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[22]. Found in all parts of the plant, the latex is usually poisonous and probably developed in order to protect the plant from herbivores.

Cassia occidentalis also called coffee weed is a small tree that belongs to the phylum: tracheobionta, class: magnoliopsida, order: fabales, family: Fabaceae, genus: *Cassia* and Specie: *Occidentalis*. The specie gives off a foul odour when damaged. It is indigenous to Brazil, it is also found in warmer climates and tropical areas of south Central and north American. In East Africa, it is commonly known as ant bush, arsenic bush or Negro coffee. In Nigeria, it is known as Nigerian senna or stinking weed[24], Akidi ogbara by the Igbos, Dora rai by the Hausas and Aboo rere by the Yorubas[25].

The motivation behind this study was to explore the possibility of formulating a natural insecticide of botanical origin that can effectively replace the more toxic synthetic insecticides currently in use. The objective was to expose albino rats to different concentrations of essential oils extracted from the leaves of *E. milii* and *C. occidentalis* with the view to ascertain the effect of these oils on biochemical markers of liver and kidney toxicity.

MATERIALS AND METHODS

This study was carried out at the Chemical Pathology Unit of the University of Calabar Teaching Hospital, Ezeagbor, Cross Rivers state, Nigeria. It lasted for six months from October, 2017 to April, 2018.

Equipments: 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 Cole-Parmer Instrument Co., USA. Auto Chemical analyzer, Selectra Pro 5 Elitech Clinical Systems, France. Echotherm Heating dry bath (Toney Pines Scientific 2713 Loker Avenue West Carlsbard. CA29010. Model IC20XR. SERIAL NO: 08021302 USA.

Reagents and chemicals: All reagents and chemicals used were of analytical grade, they include; n-hexane, AST, ALP, ALT, albumin, urea and creatinine assay kits (ELI Tech Clinical Systems SAS-Zone Industrielle-61500 SEES FRANCE.

Collection and identification of plant samples: The *E. milii* and *C. occidentalis* plants were earlier identified by a botanist in the Department of Biological Sciences (Botany), College of Natural Sciences, Michael Okpara University of Agriculture, Umudike Abia state. Leaves were thereafter harvested in the desired quantity from No 4 Edim Otop close, off victory way, Satellite town Calabar, Cross Rivers state. The plants appeared healthy, leaves bright green in colour and flowers intact at the time of the harvest.

Experimental animals: A total of 28 rats were used. Rats were monitored from birth and separated from male rats at 4 weeks before attaining sexual maturity. They were adult female nulliparous and non-pregnant weighing between 130 and 150 g. The rats were 8 weeks old as at the time of this research and weighed between 150 and 175 g. The animal ethics procedures were complied with during the whole experimental process as 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. Rats were fed standard rat chow and tap water *ad libitum* with regulated temperature and humidity and a 12/12 h light-dark cycle.

Soxhlet extraction of oils from plants: The dried leaves (dried for a period of 2 weeks under shade) of *E. milii* and *C. occidentalis* were pulverized using an electric blender, put in an air-tight container and used subsequently for soxhlet oil extraction. 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[26]. For each 50 g of ground leaves processed, 4 g equivalent of *E. milii* oil and 5 g of *C. occidentalis* oil was obtained, this amounts to 8 and 10% oil yield, respectively. The procedure was run four times to obtain 16 g of *E. milii* and 20 g of *C. occidentalis* used for the study.

Effect of plant oils on biochemical indices of rats: Exactly 5000 mg kg^{-1} b.wt., of each extract was used as the limit dose; this dose was selected based on previous studies conducted by Silva *et al.*[27]. *Cassia occidentalis* showed LD_{50} higher than 5000 mg kg^{-1}. While *Euphorbia schimperiana*; a plant in the Euphorbia family, at a limit dose of 5000 mg kg^{-1} b.wt., caused no mortality or clinical signs of toxicity during fourteen days post dose[28]. Rats were sacrificed under light ether anaesthesia. Twenty eight rats were divided randomly into 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, 3000 and 5000 mg kg^{-1} b.wt., of *C. occidentalis* oil extract, while groups C_1, C_2 and C_3 received 1500, 3000 and 5000 mg kg^{-1} b.wt., of *E. milii* oil extracts. The administrations were done orally once a week for 2 weeks.

Blood sample collection for serum biochemical analysis: At the end of 2 weeks, rats were fasted overnight and sacrificed under light ether anaesthesia. Blood samples were drawn via cardiac puncture using a 2 mL sterile syringe. About 1 mL of blood was collected and dispensed into plain test tubes which were allowed to stand for complete clotting. The clotted blood was centrifuged and the serum aspirated. Determination of urea, creatinine, albumin, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were done using biochemical assay kits manufactured by ELI Tech Clinical Systems SAS-Zone Industrielle-61500 SEES France.

Determination of serum biochemical indices: The colorimetric determination of albumin was done by the method of Doumas *et al.*[29]. The ALT activity was determined by the method already reported by Henley[30]. The AST activity according to the method of Bergmeyer *et al.*[31]. Serum creatinine concentration was determined according to the method previously published by Toro and Ackermann[32]. Serum ALP activity was determined according to the method of Haussament[33]. While urea was determined according to the Berthelot method as described earlier by Fawcett and Scott[34] and modified by Patton and Crouch[35].

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).

RESULTS

Biochemical indices of rats administered *E. milii* Oil: In Table 1, a significant ($p<0.05$) decrease in ALT activity, creatinine and urea concentrations and increase ($p<0.05$) in ALP and AST activities relative to the control at 3000 and 5000 mg kg^{-1} b.wt., was observed.

Biochemical indices of rats administered *C. occidentalis* oil: In Table 2, significant ($p<0.05$) decrease in albumin, creatinine, urea concentrations and ALT activity and increase ($p<0.05$) in AST and ALP activities relative to the control was observed at 5000 mg kg^{-1} b.wt.

DISCUSSION

Results from this study showed that oil extracts from the leaves of *E. milii* and *C. occidentalis* may be safe for use by humans as insecticidal agents, with its toxicity manifesting only at very high concentrations (3000 and 5000 mg kg^{-1} b.wt). No significant ($p>0.05$) change in most biochemical parameters was observed at 1500 mg kg^{-1} b.wt., indicating that these extracts may be safe for insecticidal application by man. This agrees with the report of Nuhu and Aliyu[36], who reported slight toxicity in the use of crude extracts from *C. occidentalis* as concotion for liver ailments. Another researcher also reported that methanolic extracts of *Euphorbia schimperiana*, a plant in the same genus, exhibited slightly toxic effects on mice[28].

Elevated serum levels of alkaline phosphatase (ALP) activity as observed in this study have been associated with chronic kidney disease (CKD)[37]. While elevated aspartate transaminase (AST) activity is linked to; liver disease or injury, ischemic/toxic damage to the liver, acute viral hepatitis, medications, acute biliary obstruction and alcohol abuse[38,39].

SWAN (a synthetic insecticide) caused significantly lower body weight gain and produced; diarrhoea, loss of appetite, eye discharge and mortality even at a low dose[40] of 5 mg kg^{-1} day^{-1} *E. milii* and *C. occidentalis* did not cause any mortality even at 5000 mg kg^{-1} b.wt., per week. This agreed with the report of a researcher that acute and sub-acute administration of hydroalcoholic extract of

Table 1: Biochemical indices of rats administered *E. milii* oil

Parameters	Albumin (g dL^{-1})	ALT (IU L^{-1})	AST (IU L^{-1})	ALP (IU L^{-1})	Creatinine (μmol L^{-1})	Urea (μmol L^{-1})
Control	4.18±0.32	21.58±2.40	92.65±0.38	108.35±0.72	59.60±9.04	7.50±0.20
1500 mg kg^{-1}	3.83±0.03	20.23±2.31^{a,b}	94.28±0.43^{b}	110.58±1.95*	51.43±0.54*^{,a,b}	4.75±0.03*^{,a,b}
3000 mg kg^{-1}	3.80±0.04	11.98±0.25*	97.93±1.26*^{,a}	112.50±1.24*	43.63±0.54*	5.30±0.06*^{,a}
5000 mg kg^{-1}	3.80±0.04	14.95±0.50*	93.33±1.01	115.33±1.06*	33.83±3.87*	4.70±0.04*

Values are expressed as the Mean±SEM (n = 4), *Significantly different from control at p = 0.05, [a]Significantly different from 1500 mg kg^{-1} at p = 0.05, [b]Significantly different from 3000 mg kg^{-1} at p = 0.05

Table 2: Biochemical indices of rats administered *C. occidentalis* oil

Parameters	Albumin (g dL^{-1})	ALT (IU L^{-1})	AST (IU L^{-1})	ALP (IU L^{-1})	Creatinine (μmol L^{-1})	Urea (μmol L^{-1})
Control	4.18±0.32	21.58±2.40	92.65±0.38	108.35±0.72	59.60±9.04	7.50±0.20
1500 mg kg^{-1}	3.70±0.08	21.05±1.75^{a,b}	94.40±0.94	109.33±0.95^{a}	39.03±3.17*	5.08±0.06*^{,a}
3000 mg kg^{-1}	4.23±0.10^{a}	14.78±1.86*	96.30±0.49*	112.18±2.39^{a}	39.08±4.77*	5.40±0.06*^{,a}
5000 mg kg^{-1}	3.53±0.15*	11.38±1.60*	96.43±1.02*	139.30±11.37*	56.58±5.29	4.70±0.13*

Values are expressed as the Mean±SEM (n = 4), *Significantly different from control at p = 0.05, [a]Significantly different from 1500 mg kg^{-1} at p = 0.05, [b]Significantly different from 3000 mg kg^{-1} at p = 0.05

C. occidentalis is not toxic in male and female Wistar rats suggesting safety for use by humans[27]. According to their report, the extract did not produce any hazardous symptoms or death in the acute toxicity test showing LD_{50} higher than 5 g kg^{-1}. In yet another study by Isah *et al.*[41], sub-acute administration of up tp 3000 mg kg^{-1} b.wt., of aqueous leaf extract of *Cassia coccidentalis* did not produce significant effects on kidney of albino rats thereby suggesting a non-lethal effect on kidneys. In the current study however, even though the oils did not cause any significant ($p<0.05$) change in kidney function markers at 1500 mg kg^{-1}, it caused a significant ($p<0.05$) decrease in urea and creatinine concentrations at 3000 mg kg^{-1} b.wt. Low levels of creatinine in the body could be a sign that the liver or muscle is not working properly[42]. This could be caused by muscle diseases such as muscular dystrophy; characterized by muscle weakness, muscle stiffness, pain and decreased mobility. Since it is produced in the liver, an unhealthy liver will be unable to make the normal amount of ceatinine[42]. Liver diseases arising from; poor liver function, interference with creatine production can also cause low creatinine concentration[43]. Reduced plasma/serum urea can be caused by decreased urea production, urinary urea excretion or a combination of the two[44]. Pathological cause of reduced urea concentration is largely confined to advanced liver disease[45]. This simply meant these oils may cause liver and kidney damage at very high concentrations. This agreed with the report that crude latex of *E. milii* caused a higher frequency of minor malformations only at very high doses which are embryolethal and maternally toxic. They concluded that this plant possess no teratogenic hazard or at least the possibility is of a considerably low order of magnitude[46].

The significant ($p<0.05$) decrease in ALT activity corroborated with a previous study which revealed that *C. occidentalis* extract significantly reduced serum levels of ALT suggesting hepatoprotection of extracts[47]. While the significant ($p<0.05$) decrease in albumin concentration at 5000 mg kg^{-1} of *C. occidentalis* oil may indicate; liver failure, heart failure, kidney damage, protein losing enteropathy, malnutrition or chemotherapy[48]. However given the low concentrations of active compounds usually required for insecticidal action on pests, (for instance SWAN; a synthetic insecticide, contains 0.45% tetra-methrin and 0.15% beta-cypermethrin) these oils may be considered safe alternatives to the harmful insecticidal chemicals currently in use.

CONCLUSION

The oils may be considered safe for use as insecticidal agents since toxicity indices are only manifested at very high concentrations. They could gradually replace the more toxic synthetic chemicals in the management of pests and disease vectors. Farmer in the rural and urban settings should be encouraged to embrace the use of natural insecticides to replace the more toxic synthetic ones in order to improve life and increase longevity.

SIGNIFICANCE STATEMENT

This study discovered the safety of plant oils that could be beneficial for agricultural pest control and increased productivity and economic growth. This study will help the researcher to uncover the critical areas of biopesticide toxicity that many researchers were not able to explore. Thus a new theory on essential oils as biopesticides may be arrived at.

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