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Research Article
Insecticidal Oils from the Leaves of *Cassia occidentalis* and *Euphorbia milii* May Induce Hormonal Changes in Rats

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Abstract
Background and Objectives: The need for natural alternatives to the toxic synthetic chemicals currently used for the control of insect pests and vectors inspired this research. This study analyzed the effect of insecticidal oils obtained from the leaves of *Cassia occidentalis* and *Euphorbia milii* on some reproductive hormones in rats. **Materials and Methods:** Oils were extracted from both plants via soxhlet extraction and administered to rats orally once a week, for 2 weeks. 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, while groups C_1, C_2 and C_3 received 1500, 3000 and 5000 mg kg^{-1} b.wt., of *E. milii* oil. Rats were thereafter sacrificed under light ether anaesthesia. **Results:** *Euphorbia milii* oils caused a significant ($p<0.05$) increase in the concentrations of testosterone, estradiol and luteinizing hormone at 3000 and 5000 mg kg^{-1} b.wt., relative to the control. There was also a significant increase ($p<0.05$) in luteinizing hormone and testosterone concentrations in rats administered *C. occidentalis* oil at 3000 and 5000 mg kg^{-1} b.wt., relative to the control. However, no significant ($p>0.05$) change in estradiol concentration was observed with *C. occidentalis* oil extract. There was no significant change ($p>0.05$) in the concentration of most hormones at 1500 mg kg^{-1} b.wt.. **Conclusion:** Since insecticides are usually applied at much lower concentrations, the plant oils may be considered safe to reproductive hormones in animals.

Key words: Insecticidal, *Euphorbia milii*, Hormonal, *Cassia occidentalis*, Reproductive

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

INTRODUCTION

The importance of developing organic insecticides for pest control has been continuously emphasized, as it is assumed that natural insecticides present less risk to the environment than their synthetic counterparts[1]. Among the biopesticides, botanicals are presently at the fore-front due to the eco-toxicological properties of the non-botanicals. Higher plants are rich sources of novel natural substances that can be used to develop environmentally safe products for insect control[2]. Oils extracted from the leaves of *E. milii* and *C. occidentalis* has been reported to possess reasonable levels of insecticidal efficacy against insect pests[3]. The effect of these oils on reproductive hormones has however, not been reported.

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[4]. 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 where they penetrate groundwater, pollute streams and harm wildlife[5].

Hormones travel from their point of release in the bloodstream to particular tissues where they convey their messages. For the message to be interpreted, hormones bind to receptors. Hormone and receptor have a precise fit, so that only a specific type of hormone can bind to a specific receptor[6]. A number of environmental agents may alter this process by mimicking the natural hormone (agonists) or by inhibiting receptor binding (antagonists). The latter mechanism is based on complete or partial blocking of the specific receptor. At the environmental level, wildlife is particularly vulnerable to the endocrine disrupting effects of some synthetic pesticides. Effects linked to endocrine disruption have been largely noted in invertebrates, reptiles, fish, birds and mammals[7]. Most of them are linked to exposure to organochlorine pesticides (OC) and affect the reproductive function. Biological monitoring studies indicate that pesticide exposures are widespread in the human population. Dietary exposure comes from residues in fruits, vegetables and from contaminated meat, fish, rice and dairy products[8].

Several pesticides or their metabolites have been reported to possess estrogenicity *in vivo*, e.g., methoxychlor[9]. Pesticides that may disrupt the hormonal function are often called endocrine disrupting chemicals (EDCs) just like other agents with similar mechanisms of action[10]. An ECD may be defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body. They are responsible for the maintenance of homeostasis, reproduction, development and/or behaviour[11]. Endocrine disruptors are usually either natural products or synthetic chemicals that mimic, enhance (agonists) or inhibit (antagonists) the action of endogenous hormones[11]. There are indications that certain pesticides can influence the hormonal balance and thus increase the risk of subfertility[10].

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* and specie: *Milii*[12]. 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. 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[13]. The latex which is found in all parts of the plant 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* (https://en.wikipedia.org/wiki/Senna_occidentalis). The specie gives off a foul odour when damaged. It is indigenous to Brazil, but 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[14], *Akidi ogbara* by the Igbos, Dora rai by the Hausas and Aboo rere by the Yorubas[15]. The purpose of this study was to determine the impact of Insecticidal oils from the leaves of *C. occidentalis* and *E. milii* on some reproductive hormones in rats.

MATERIALS AND METHODS

The study was carried out at the Endocrinology Laboratory of the department of Biochemistry, University of Calabar, Cross Rivers State, Nigeria. It lasted from October, 2015 to September, 2017.

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. Incubator Hirayama Manufacturing Corporation Japan, Spectrophotometer (Beckman Coulter, DU 520 general purpose UV/Visual).

Biochemicals and chemicals: All chemicals used were of analytical grade they include; Estradiol (E2), testosterone and luteinizing hormone test systems (Monobind Inc, Lake Forest, CA 92630, USA. Elitrol 1 (normal control) and Elitrol II (abnormal control).

Collection and identification of plant samples: *Euphorbia milii* and *C. occidentalis* plants were identified by a botanist in the Department of Biological Sciences (Botany), College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State and deposited at the Herbarium Unit of the Department of Biological Sciences (Botany) University of Calabar, Cross Rivers State, Nigeria. Voucher No: 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. The leaves appeared healthy, bright green in colour with intact flowers at the time of 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 of *E. milii* and *C. occidentalis* were pulverized using an electric blender into a fine powder. 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[16].

Experimental design: Rats were randomly divided 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. The limit dose was selected based on previous studies conducted by Silva *et al.*[17] and Ahmed *et al.*[18].

Blood sample collection for hormonal profile 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 was dispensed into plain test tubes and allowed to stand for complete clotting. The clotted blood was centrifuged and the serum aspirated. Serum concentrations of, estradiol, testosterone and luteinizing hormone were determined.

Determination of serum hormonal indices: Estradiol was determined according to the method described by Abraham[19]. Testosterone was determined by the method of Castro *et al.*[20] while Luteinizing hormone was determined by the method described by Kosasa[21] using ELISA (Estradiol (E2), testosterone and luteinizing hormone test systems manufactured by Monobind Inc, Lake Forest, CA 92630, USA.

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 AND DISCUSSION

Cassia occidentalis oil caused a significant ($p<0.05$) increase in luteinizing hormone and testosterone at 3000 and 5000 mg g kg^{-1} b.wt. No significant ($p>0.05$) change in estradiol was observed at all concentrations of *C. occidentalis* administered to animals.

Euphorbia milii oil caused significant ($p<0.05$) increase in all tested hormones at 3000 and 5000 mg kg^{-1}/b.wt., relative to control. However, no significant $p>0.05$ change was observed in hormones at 1500 mg kg^{-1}/b.wt.

Table 1: Effect of oil from *C. occidentalis* on some hormones in rats

Groups	LH (mIU L^{-1})	Testosterone (g mL^{-1})	Estradiol (pg mL^{-1})
Control	10.35±0.42	0.53±0.02	1235.21±2.46
1500 mg kg^{-1}	12.51±0.10*	0.53±0.11	1235.75±0.55
3000 mg kg^{-1}	13.02±0.20*	0.54±0.01*[,a]	1236.75±0.75
5000 mg kg^{-1}	12.83±0.10*	0.55±0.01*[,b]	1235.75±0.55

Values are expressed as the Mean±SEM, n = 4, *Significantly different from control at $p<0.05$, [a]Significantly different from 5000 mg kg^{-1} at $p<0.05$, [b]Significantly different from 3000 mg kg^{-1} at $p<0.05$

Table 2: Effect of oil from *E. milii* on some hormones in rats

Groups	LH (mIU L^{-1})	Testosterone (g mL^{-1})	Estradiol (pg mL^{-1})
Control	10.35±0.42	0.53±0.02	1235.21±2.46
1500 mg kg^{-1}	10.79±0.02[a,b]	0.51±0.02[a,b]	1235.00±0.82[a,b]
3000 mg kg^{-1}	12.45±0.07*[,a]	0.63±0.05*[,a]	1239.75±0.73*
5000 mg kg^{-1}	11.97±0.26*[,b]	0.57±0.10*[,b]	1240.75±0.85*

Values are expressed as the Mean±SEM, n = 4, *Significantly different from control at $p<0.05$, [a]Significantly different from 5000 mg kg^{-1} at $p<0.05$, [b]Significantly different from 3000 mg kg^{-1} at $p<0.05$

Oil extracts from both plants caused significant ($p<0.05$) increase in luteinizing hormone and testosterone concentrations at 3000 and 5000 mg kg^{-1} b.wt., relative to the control. However, *C. occidentalis* oil did not cause any significant change in estradiol at all concentrations administered (Table 1, 2).

This means that in animals, the plant extracts may affect reproductive abilities at very high concentrations (3000/5000 mg kg^{-1} b.wt.). In a study by Idrovo *et al.*[22], women who worked with pesticides had a 60-100% increased odds of experiencing long cycles, missed periods and intermenstrual bleeding compared with women who had never worked with pesticides. In another study, an increased risk of infertility was observed among women exposed to pesticides or working in industries associated with agriculture[23]. Hormonal balance is important to preserve female reproduction and maintain fertility. This balance can be disturbed by changing levels of estrogen or progesterone. If one substance or link is disturbed in the chain of hormone synthesis, the hormone may not be produced or may get different properties. Though the effects of organophosphorus (OP) pesticides on the human hormonal profile have not yet been fully characterized, differences in serum hormone levels were observed to be according to the severity of exposure[24]. Treatment of rats with the insecticide heptachlor suppressed estradiol concentrations in blood and reduced the production of estradiol by ovarian cells of treated rats[10]. In this study however, *C. occidentlais* oil did not cause any significant change ($p>0.05$) in estradiol at all concentrations tested relative to the control (Table 1), while *E. milii* oil at 3000 and 5000 mg kg^{-1} b.wt., significantly increased estradiol concentration in rats.

The significant ($p<0.05$) increase in testosterone and luteinizing hormone (LH) concentrations observed with *C. occidentalis* at 3000 and 5000 mg kg^{-1} b.wt., in rats relative to the control. Table 1 shows that at high concentrations, *C. occidentalis* could affect the development of sexual secondary characteristics as well as reproduction. This agrees with the report of Sarkar *et al.*[24], who reported that chronic exposure to sublethal doses of quinalphos increased LH, prolactin and testosterone serum levels in rats, whereas, exposure to dimethoate decreased LH serum levels in sheep[25]. Serum testosterone levels were significantly higher in individuals employed at companies whose production methods were not organic, when compared with the group of workers using organic methods[26]. Studies in animal models have reported that OP pesticides may act as endocrine disruptors. In rats, chlorpyrifos-methyl showed anti-androgenic activity, probably mediated by binding to androgen receptors[27].

Increased levels of LH in females connote a problem with the ovaries. High LH levels in men can indicate primary testicular failure which is caused by; trauma, history of taking chemotherapy medications, a tumour in the central nervous system, brain injury, inflammation or infection in the central nervous system, such as meningitis or encephalitis or a history of brain surgery[28]. While 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 of 5 mg kg^{-1}/day[29]. *Euphorbia milii* and *C. occidentalis* did not cause any mortality at 5000 mg kg^{-1}/week. Given the small concentration usually required for insecticidal control (43.7-1000 ppm) which may vary depending on environmental factors and plant species[30], oils may be considered safe for use as insecticidal agents. This study suggests that the plant oils are much safer to mammals than their synthetic counterparts and may be used effectively as insecticidal agents to replace the more toxic synthetic ones.

CONCLUSION

While these plant oils have proven to affect reproductive hormones in rats, they do so only at very high concentrations. Since their toxic effects are only manifested at extremely high concentrations; they may effectively replace the more toxic synthetic insecticides. Farmers should be protected from harsh chemicals that damage their body systems and reduce their life span, by encouraging the use of natural pesticides.

SIGNIFICANT STATEMENT

This study discovered the insecticidal oils from *C. occidentalis* and *E. milii* that can be beneficial for the control of insect pests and vectors. This study will help the researcher to uncover the critical areas of endocrine toxicity that many reseachers were not able to explore. Thus a new theory on the safety of insecticidal botanical oils on reproductive hormones may be arrived at.

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