Effects of *Jatropha gossypifolia* leaf Extract on the Reproductive Cycle of Cypermethrin-treated Female Rats

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**ABSTRACT**

Cypermethrin (Cyp), a type II pyrethroid, is a broad-spectrum insecticide extensively used for pest management and animal husbandry practices. They have been named among the endocrine disrupting chemicals (EDCs). Previous studies have reported its adverse effect on reproduction with no detailed information on the effect on female reproductive cycle. Methanolic extract of *Jatropha gossypifolia* leaves was investigated for its attenuative effects on disrupted oestrous cycle and hormones of cypermethrin-treated female wistar rats. Thirty-two female albino rats (180.23 ± 3.21 g) were completely randomized into four groups (A–D) of eight animals each. Animals in group A served as the control and received 0.5 ml of corn oil. Animals in groups B, C and D received 20 mg/kg body weight (BW) of cypermethrin and treated with 0.5 ml of corn oil, 50 and 100 mg/kg BW of *Jatropha gossypifolia* extract (JGE) respectively daily for 30 days and sacrificed 24 hours after the last administration. The oestrous cycle was monitored by vaginal cytology between 09:00 and 10:00 hours throughout the exposure period. Four reproductive hormones were assayed for in the serum of the animals: Progesterone (P), Testosterone (T), Luteinizing hormone (LH) and Follicle stimulating hormone (FSH). The oestrous cycle was irregular in female animals given cypermethrin and distilled water. The pattern of irregularity involves persistent/extended estrus phase compared to the control group. The administration of 50 and 100 mg/kg BW of JGE attenuated the effect of cypermethrin by reversing the irregularity in the oestrous cycle. The administration of JGE attenuated the effect of cypermethrin on serum testosterone, progesterone, FSH and LH concentrations. The phytoconstituents in the plant might be responsible for the attenuative benefits of *Jatropha gossypifolia* leaves in the management of cypermethrin-induced alterations of the reproductive cycle.

**Keywords:** Cypermethrin, *Jatropha gossypifolia*, oestrus cycle, hormones, vaginal cytology.

**INTRODUCTION**

Cypermethrin is an insecticide in the synthetic pyrethroid family commonly used to kill household insect pests. It was first synthesized in 1974 and first marketed in 1977 [1]. Aside organophosphate pesticides, cypermethrin is the most commonly used pyrethroid [2]. Cypermethrin, like all synthetic pyrethroids, kills insects that eat or come into contact with it by disrupting normal functioning of the nervous system [3]. Humans exposed to cypermethrin show symptoms which include fatigue [4], headaches, loss of bladder control [3], muscle twitching as well as seizures [8] while laboratory animals exhibit features such as burrowing, seizures, tremors and writhing [6, 7]. Cypermethrin could also disrupt the normal functioning of sex hormones [8]. Studies have shown that cypermethrin reduced growth rate and increased liver weight in rats [9], causes anemia in mice; loss of appetite and tremor in dogs [1] and pathological changes in vital organs of rabbits [3].

Traditional medicine using extracts from plants have provided health coverage for a large percentage of mostly the developing world with *Jatropha gossypifolia*, a member of the eurphorbiaceae family, playing a key role [9]. Widely known as “bellyache bush”, other common names of *Jatropha gossypifolia* are pignut and fignut. In Yoruba land it is commonly known as “Lapalapa pupa” [10]. Traditional medicine in Latin America and Africa use this plant species in the treatment of several ailments and/or as ornamental crops [9]. Among other local uses of this plant, various reports have shown that thrush could be treated with the young stem of *Jatropha gossypifolia*. Scientific reports on the medicinal uses of *Jatropha gossypifolia* include antipyretic, antimicrobial and antianeamic properties among others [9, 11, 12]. All these properties could be attributed to the presence of some secondary metabolites such as flavonoids, alkaloids and phenols that have been detected in various extracts from different parts of the plant [13, 14].
MATERIALS AND METHODS

Plant material

Fresh leaves of *Jatropha gossypifolia* was collected at Opa street, Ile-ife, Osun State, Nigeria. The plant material was submitted to the Department of Plant Science in Ekiti State University, Ado-Ekiti for identification and authentication. The electronic herbarium number UHAE163 was then assigned to it.

Methanolic Extraction of *Jatropha gossypifolia*

Fresh leaves of *J. gossypifolia* were harvested, rinsed in clean water and spread under the shade on a clean dry surface to air dry for about two weeks. It was then grinded into fine powder using a blender. Exactly 388 g of the powdered leaves was measured into a conical flask and soaked with 3.2 litres of methanol. The mixture was allowed to stand for 72 hours with periodical shaking. The solvent was then decanted and sieved using Whatman No 1 filter paper. The filtrate was subjected to evaporation in a water-bath at 45°C to obtain the viscous extract which was stored at -4°C until use. Percentage yield of the methanolic extract obtained was 10.57% w/w.

Equipment and Reagents

All chemicals used in this study were of analytical grade and were used without further purification. Methanol were from Qualikems Laboratory reagent while cypermethrin (10% EC) (trade name: Cyperforce) was obtained from an agricultural store in Abeokuta, Nigeria. Whole blood samples were collected using capillary tubes into lithium heparin sample bottles prior separation. The separations were performed using ultra-centrifuge (Thermo Scientific Sorvall M150 SE) set at 4000rpm for 10 minutes after which the supernatant was aspirated into a plain sample bottle and kept in the deep freezer until analysis. Cells and samples on slides were viewed under the light microscope (Olympus C5) and Portable Digital Microscope.

Experimental Animals

Thirty-two (32) female albino rats weighing between 150-200g were purchased from the animal house of Olu Research Animals located at Ibadan, Nigeria. The animals were kept in the animal house of the Department of Biological Sciences, Wesley University Ondo, Nigeria. They were subjected to a period of acclimatization for two weeks with an approximately twelve hours of light and dark cycle. The animals were fed a standard laboratory feed and water ad libitum for a period of sixty days. This was immediately followed by sixty days of cytology and thirty days of administration. The animals were given 0.5 ml of the stock solution via oral administration using orogastric canula based on their weight ranges and dose for a period of thirty days. The animals were completely randomized into four groups (A-D) of 8 animals each. Animals in group A served as the control and received 0.5 ml of corn oil. While those in groups B received only cypermethrin (20 mg/kg BW). Animals in groups C and D received similar dose of cypermethrin with 50 and 100 mg/kg BW of *Jatropha gossypifolia* extract (JGE) respectively on daily basis for 30 days and sacrificed 24 hours after the last administration. The oestrous cycle was monitored by vaginal cytology between 09:00 and 10:00 hours throughout the exposure period. Testosterone (T), Follicle stimulating hormone (FSH), Luteinizing hormone (LH) and Progesterone (P) were assayed for in the serum of the animals.

Blood Collection

Blood samples were collected with the aid of heparinized capillary tubes through ocular puncture into labelled heparinized sample bottles. These samples were then analysed using an auto analyzer in Central Diagnostic Laboratory, Tanko, Ilorin, Kwara state, Nigeria.

Cytology

Female wistar rats of about three months old (150 to 200 g) were used for this study. During the experimental period, light is always on at 6 a.m. under a controlled temperature room (22-25°C), with a 12 hours light and 12 hours dark cycle. The vaginal secretion was collected with the aid of cotton bud everyday throughout the experimental period between the hours of 8 and 9 am. The tip of the cotton bud is dipped in 10 ml of normal saline (NaCl, 0.9%) to moisten it prior to insertion into the rat vagina, but not deeply. The vaginal secretion on the bud is smeared on microscope slide and observed under a light microscope at a magnification of 400x. Epithelial cells (round and nucleated), cornified cells (irregular and without nucleus) and leukocytes (the little round ones) are the three types of cells identified in the course of this study and their proportion was employed in the determination of the rats’ oestrous cycle phases.3

Statistical Analysis

Statistical package for social sciences (SPSS – 17) was employed and the percentage organ to body weight ratios as well as the rats’ body weights were expressed as mean ± SD. Comparison of values within the groups was done using the analysis of variance (ANOVA) with the level of statistical significance fixed at p<0.05 at 95% confidence interval for all analyses.

RESULTS

Daily vaginal cytology of cypermethrin-treated animals administered corn oil revealed an alteration in the consistent 4 -5 days of estrous cyclicity evident by the persistent presence of cornified epithelial cells as compared to the control which then prolonged the oestrous cycle from the normal 4 days cycling to 8-9 days (Plate 1). The doses of 50 and 100 mg/kg body weight of JGE reversed the trend of irregularity in oestrous cycle of the cypermethrin-treated rats in a manner similar to the control animals thereby attenuating the effect of cypermethrin by reversing the irregularity in the oestrous cycle. Furthermore, leukocytes, epithelial cells and cornified cells were seen in the vaginal smears of cypermethrin-treated animals given the various doses of JGE (Plate 1-4).

The testosterone concentration of animals administered cypermethrin was significantly (p<0.05) increased when compared with the control animals (Figure 1). The administration of cypermethrin to female rats significantly (p<0.05) decreased the progesterone and luteinizing hormone concentration when compared with the control animals (Figures 2 and 3). The doses at 50 and 100 mg/kg body weight of JGE to cypermethrin-treated animals did not alter the concentration of testosterone, progesterone and luteinizing hormone significantly (p>0.05) when compared with the control animals (Figures 1, 2 and 3).

The concentration of FSH in animals administered with cypermethrin was not significantly (p>0.05) altered compared with control animals (Figure 4). The administration of 50 mg/kg BW of JGE significantly (p<0.05) decreased the FSH concentration of the cypermethrin-treated animals while FSH concentration of animals administered 100 mg/kg BW of JGE was not significantly (p>0.05) altered compared to the control animals (Figure 4).
Plate 1: Photomicrograph of vaginal smear of oestrus phase of the oestrous cycle of female rats showing cornified cells (C) (× 40)

Plate 2: Photomicrograph of vaginal smear of proestrus phase of the oestrous cycle of female rats showing Epithelial cells (E) (× 40)

Plate 3: Photomicrograph of vaginal smear of diestrus phase of the oestrous cycle of female rats showing Leucocytes (L) (× 40)

Plate 4: Photomicrograph of vaginal smear of metestrus phase of the oestrous cycle of female rats showing cornified cells (C), epithelial cells (E) and leucocytes (× 40)

Figure 1: Testosterone concentration of cypermethrin-treated female rats administered methanolic extract of J. gossypifolia leaf

Figure 2: Progesterone concentration of cypermethrin-treated female rats administered methanolic extract of J. gossypifolia leaf

Figure 3: Luteinizing hormone (LH) concentration of cypermethrin-treated female rats administered methanolic extract of J. gossypifolia leaf

Figure 4: Follicle stimulating hormone (FSH) concentration of cypermethrin-treated female rats administered methanolic extract of J. gossypifolia leaf
DISCUSSION

Dysfunctions in Reproductive system associated with exposure to hormonally active compounds has been archived extensively [17] as endocrine disruptors, and various chemicals have been highlighted for study in endocrine responsive bioassays [18]. This study reports on the attenuative efficacy of J. gossypifolia leaf in cypermethrin-treated rat model adopting cytological and hormonal bioassay parameters. Synthesized pyrethroids are related to natural chemical moiety, pyrethrin, isolated from the chrysanthemum [19]. Cypermethrin, a synthetic pyrethroid [19], is used as insect-killers against ticks [20], mites [21], mosquitoes [22, 23], and as treatment for lice on human head [24] and scabies [25]. Due to the high human exposure to cypermethrin usage as insecticide, the attenuative effect J. gossypifolia leaf extract on the reproductive cycle and hormones was investigated in this study. This hormonal dysfunction created by cypermethrin resulted in irregular and/or prolonged estrous cycle. The doses at 50 and 100 mg/kg body weight of JGE to cypermethrin-treated rats completely mitigated the effect of cypermethrin on the oestrous cycle and thereby resulting to the optimal concentration of FSH to cypermethrin effect on the synthesis of follicle stimulating hormone concentrations comparing favourably with the control female rats. Furthermore, the administration of 50 mg/kg body weight of J. gossypifolia leaf extract to cypermethrin-treated rats did not mitigate the effect of cypermethrin in the synthesis of follicle stimulating hormone, thus, resulting to decreased FSH concentration. In contrast, administration of 100 mg/kg body weight of J. gossypifolia leaf extract to cypermethrin-treated rats compared favourably with the control group and thereby resulting to the optimal concentration of FSH concentration.

The findings in the present study with respect to oestrous cycle, T, P, LH and FSH serum concentrations agrees with the work of Colborn et al. [17] which reported that cypermethrin causes reproductive dysfunctions by serving as endocrine disruptors. The presence of bioactive agents detected in different extracts from different parts of the plant such as alkaloids, coumarins, phenols, aponins, lignoids, flavonoids, steroids, tannins, and terpenoids in J. gossypifolia leaf extract as reported by Zhang et al. [13] and Faokunla et al. [14] might be responsible in mitigating the effect of cypermethrin. For example, the presence of alkaloids in J. gossypifolia leaf extract may not be unconnected with the pro-progestogenic activity displayed by the botanical. These phytochemicals in J. gossypifolia leaf extract may be responsible for its attenuative effect on the reproductive cycle and sex hormones in cypermethrin-treated female rat.

CONCLUSION

In conclusion, methanolic extract of J. gossypifolia leaf not only attenuated cypermethrin effect on the reproductive cycle but also mitigated its effect on testosterone, progesterone, LH and FSH concentrations as investigated in the present study.

REFERENCES


HOW TO CITE THIS ARTICLE