Dicliptera laxata (Acanthaceae) (C. B. Clark) Unblocks an Abamectin-Blocked Estrous Cycle and Restores Cyclicity in Female Albino Rats

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ABSTRACT

The farmers of the Buea municipality, Fako Division, South-West Region, Cameroon extensively use Abamectin or Aba, a phytosanitary insecticide which targets insects such as mites, thrips species and leaf miners. In this area, the local population also uses Dicliptera laxata traditionally as a fertility enhancer and in stabilizing risky pregnancies in women. This study was aimed at evaluating the actions of Dicliptera laxata aqueous extract on the estrous cycle and reproductive system of Aba-treated female rats. A total of 30 adult female rats of the confirmed regular cycle were daily treated orally with 2 ml/kg Abamectin for a period of 45 days, accompanied by the evaluation of the cytology of their vaginal smears at 5 days intervals. From the 46th day, they were subsequently treated with the leaf-aqueous extract of Dicliptera laxata for 15 days and were terminated on the 61st day during which blood and reproductive organs were collected for biochemical assays and relative weight indices. Chronic (45 days) exposure of normal cycled female rats to Abamectin witnessed a blockade in their estrous cycle, a significant drop in plasma estradiol and progesterone concentration, and a significant drop in the percentage weight of organs. Administration of Dicliptera laxata leaf-aqueous extract to these Aba-treated females resulted in a return to the regularity of their estrous cycle, with animals receiving the 300 mg/kg dose of plant extract recording a 60% regulation in the first week and 100% in the second week as compared to animals treated with distilled water that recorded a 0% and 20% regulation in the first and second week of treatment, respectively. Also, the animals that received 300 mg/kg body weight of the extract registered significant (p < 0.05) rise in plasma concentrations of progesterone and estradiol (36.44 ± 1.69 pg/mL and 77.73 ± 3.82 pg/mL, respectively) as compared to animals that received distilled water which showed 22.54 ± 2.19 pg/mL plasma progesterone and a 44.90 ± 7.63 pg/mL plasma estradiol concentrations, respectively. The actions of the leaf-aqueous extract of D. laxata could be attributed to the presence of phytochemicals including steroids, phenolic compounds, alkaloids and saponins in our extract, which have been reported to have important pharmacological effects on female reproduction. D. laxata, therefore, constitutes a potential treatment of female infertility and our findings justify its folkoric use by the Bakweri tribe in enhancing fertility. It can be used to stabilize risky pregnancies and prevent miscarriages.

Keywords: Abamectin, albino rats, D. laxata, Female infertility.
1. Introduction

The use of pesticides has increased over the past 2 decades, especially in low-income countries including Cameroon, Ethiopia and Burkina Faso, where this increase is about 8 to 50 times (Pretty & Bharucha, 2015; Tambe et al., 2019). Many farmers in these areas use these products to either repel the pests or destroy them. Unfortunately, there is over-use and misuse of pesticides resulting from the common availability of pesticides in the local markets, farmers and salesmen’s perception of pesticide rates, low level of education and inappropriate training of farmers (Chen et al., 2018; Dopavogui et al., 2022; Pouokam et al., 2017; Bertrand et al., 2016) which according to London and Baille (2001), Matthews et al. (2003), McCauley et al. (2006), and Tarla et al. (2013) are the key pesticide-exposure risk factors. Furthermore, the presence of pesticide residues in most consumed foodstuffs (Gimou et al., 2008), drinking water contamination (Sonchieu et al., 2010; Kamga et al., 2013; Tandi et al., 2014; Tarla et al., 2015) and non-use of personal protective equipment is the misuse and many risk factors that can lead to serious health risks for both farmers and the general population. These practices lead to intoxication of the body with pesticide which can be immediately or a long time after being exposed to the product. Intoxication with a pesticide may happen at home while working on the farm, accidentally ingesting the product or during mixing and spraying (Chen et al., 2018; Dopavogui et al., 2022; Matthews et al., 2003; Konradesen et al., 2003). The study of farmers’ behaviors has served as the means to better understand how their working environment, especially exposure to pesticides, affects their health (McCauley et al., 2006).

In less-developed countries, there is a rise in pesticide poisoning resulting from work (Litchfield, 2005) with an estimated 25 million agricultural workers globally experiencing unintentional pesticide poisoning, annually (Jeyaratnam, 1990). Intoxication of the body with pesticides affects a considerable part of the population in most low-income countries like Cameroon where the problem remains unaddressed since its implications are not well understood (Chen et al., 2018; Dopavogui et al., 2022; Pouokam et al., 2017; Bertrand et al., 2016).

One of the classes of pesticides is insecticides, whose toxic effects on the female reproductive system have been fully demonstrated. These substances cause a disruption in the female endocrine system and subsequent alterations in reproductive organs as well as germ cells (Kara & Öztas, 2020; Bretveld et al., 2006). Their actions disrupt the physiology of the ovary in two dimensions: alter ovarian functions causing changes in hormonal secretion and these endocrine changes, in turn, affect the female reproductive system via the Hypothalamo-Pituitary-Gonadal axis, resulting in dysfunctions. The main female dysfunctions include disruption in the biosynthesis of hormones and alteration in the maturation of follicles which lead to a disrupted ovarian cycle. Other consequences of insecticide intoxication can be a prolonged gestation period, stillbirth and infertility (Kara & Öztas, 2020; Sharma et al., 2020). In addition, insecticides could affect germ cells at primordial phases resulting in infertility at the adult stage (Sharma et al., 2020). Negative implications of insecticides in the female reproductive system have been. For example, endosulfan is known to trigger apoptosis by inducing oxidative stress in the follicle cells and also induces the expression of steroidalgenic acute regulatory proteins (StAR) CYP19A and aromatase, thus, provoking improper ovarian maturation; dichloro-diphenyl-trichloroethane (DDT) exposure inhibits CYP450-side chain cleavage enzyme, progesterone receptor, estrogen sulfotransferase, cycloxygenase-2 (COX-2) and epidermal growth factor (epiregulin) which all lead to the alteration of ovulation time (Sharma et al., 2020); chlorpyrofos alters uterine weight and morphology by causing the thickness of surface epithelium and myometrium (Kara & Öztas, 2020; Ghuman et al., 2013). And also possesses ovotoxic and embryotoxic properties while mimicking estrogen and altering embryo hatching, cell proliferation and apoptosis in zebrafish. In addition, chlorpyrifos lowers luteinizing hormone (LH), estrogen and progesterone serum levels (Nishi & Hundal, 2013; Nandi et al., 2011). Summarily, the net effect of the culminating actions of pesticide poisoning is infertility. Thus, though the world is witnessing an increase in population, some couples still remain infertile.

Infertility is a medical condition that can inflict psychological, physical, mental, spiritual, social and medical detriments to the patient. Infertility can be accompanied by feelings of shame, guilt and low self-esteem which in turn may lead to anxiety, distress, poor quality of life as well as varying degrees of depression. This medical condition is unique in its quality as it affects both the patient and the patient’s partner within a couple (Ventura et al., 2016).

Infertility is estimated to impact about 10%–25% (estimates range from 48–180 million) of couples of reproductive age (Borght & Wyns, 2018). While primary infertility is higher in other regions of the world, the secondary type is more common in Africa. The infertility rate is very high in Cameroon and it ranges from 15% to 30% depending on the age and the socio-economic level of couples. In females, it occurs in about 37% of all infertile couples with ovulatory disorders accounting for more than half of the cause of infertility (Hasanpoor-Azghdy et al., 2014).

Infertility treatment options depend on the type and its possible cause(s). Some treatment options include fertility drugs like clomiphene citrate which stimulates the pituitary gland to release more FSH and LH, thus, enhancing ovulation; Gonadotropins, such as human menopausal gonadotropin or hMG (Menopur) and FSH (Gonal-F, Follistim AQ, Bravelle) that stimulate the ovary to produce multiple eggs; Metformin (Fortamet), used in insulin resistance conditions and helps improve insulin resistance, which can increase the chances of ovulation; Letrozole (Femara), an aromatase inhibitor, has an action similar to that of clomiphene; and utrogestan, which can be taken through the oral route. Infertility can also be remedied
through surgery including laparoscopic or hysteroscopic surgery and tubal surgeries as well as through assisted conception (intrauterine insemination, assisted reproductive technology and in vitro fertilisation). These drugs have numerous side effects like bloating, headache, gastric upset, mood swings, and developing ovarian hyperstimulation syndrome. Their use is further hindered by the fact that these drugs tend to be expensive/unavailable, while surgery procedures are sometimes uncomfortable and some remote areas may lack the facilities to carry out these surgeries; hence, many women especially in developing countries suffering from infertility lack the means and finance of acquiring these treatments. These above shortcomings make individuals think of alternative ways to solve their problems which makes them rely on medicinal plants.

The use of medicinal plants either as therapeutic alternatives or food additives has run from time immemorial. For several decades, plants have been used to manage illnesses and because they are readily or cheaply available in healthcare, several decades, plants have been used to manage illnesses and because they are readily or cheaply available in healthcare (Cabada-Aguirre et al., 2023; Gwet-Bell et al., 2018). Presently, the use of medicinal and aromatic plants for the development and preparation of alternative traditional medicine and food additives has gained much interest (Bertrand et al., 2016). Medicinal plant-based drugs have possible therapeutic properties in the treatment of several diseases like infertility (Unuane et al., 2011; Hossain et al., 2013). Although other options exist for the treatment of infertility with pro-fertility drugs as mentioned above, medicinal plants still remain the best as they are relatively cheap and easily accessed with no or minimal side effects.

Abamectin or Aba (Acarius 018EC), is a phytosanitary insecticide which targets insects such as mites, thrips species, and leaf miners and is widely used by farmers within Buea municipality, found in the South-West Region of Cameroon. In this same area, Dicliptera laxata is traditionally used by the Bakweri tribe as a fertility enhancer in women. According to their oral history, its leaf/flower-aqueous extract is a potent remedy to stabilize risky pregnancies and irregular menstruation in women. However, until our study, no scientific data exists that sustains these folk claims, although its anti-inflammatory and antinociceptive (Wolde-Mariam et al., 2013), as well as anti-cancer (Ochwang’i et al., 2014) activities, have been reported. In this study, we assessed the effects of a leaf-aqueous extract of Dicliptera laxata on the Aba-blocked estrous cycle in female rats to provide a pharmacological justification for the claimed folkloric use of this plant in the treatment of female reproductive inadequacies by the people of Fako Division, South-West Region of the Republic of Cameroon.

2. Materials and Methods

2.1. Plant Material

Dicliptera laxata is a medicinal plant used traditionally by the local population in Manyu Division of Cameroon to resolve female fertility inadequacies, a claim that requires scientific investigation.

2.1.1. Collection, Identification of Plant and Extraction

Fresh leaves of Dicliptera laxata were harvested from Ntenako village, Manyu Division of the South-West Region of Cameroon under the guidance of a traditional herbalist (Besong et al., 2018) in the month of March 2022 and taken to the Cameroon National Herbarium Yaounde for identification where a voucher specimen was deposited under voucher N° 236063RF/CAM. The harvested leaves were washed thoroughly and allowed to air-dry for approximately two weeks at room temperature. The leaves were ground using an electric blender in order to produce a fine powder. Thirty-seven grams of the powder were macerated into 74 ml of distilled water and kept for 72 hours, accompanied by mechanical agitation at 24-hour intervals. This was then followed by filtration using a laboratory sieve with 8 μm pores. The filtrate was later evaporated in an oven at 40°C for a period of 5 days to obtain a 7.9 g semi-solid crude extract, giving a 21% yield of extraction (Besong et al., 2019).

2.1.2. Determination of Administrative Doses

Doses administered to the animals were obtained based on the posology used by the local traditional herbalists and folk-users; a handful of leaves (30 leaves) is macerated into about 250 ml (a glass) of water and drank twice daily for 15 days. Preliminary and screening studies revealed a 75 mg/kg body weight dose as the therapeutic dose, from which the other doses were determined.

2.2. Animals and Ethical Considerations

Animals used were female rats of the Wistar strain raised in the Animal Facility of the Department of Animal Biology and Conservation of the Faculty of Science, University of Buea under standard conditions of temperature (25 ± 1°C) and humidity (50%–80%) with a 12/12 hours light/dark cycle and in standard cages. They had free access to food and water. Each animal was used only once. All experiments were performed according to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85–23, revised 1996) and the Cameroon National Ethical Committee (Yaounde, Cameroon) for animal handling and experimental procedure (Reg No. FW-IRB00001954). Efforts were made to minimize animal suffering and reduce the number of animals used (Owumi et al., 2021). Ethical clearance was obtained from the Faculty of Science, University of Buea Institutional Animal Care and Use Committee (UB-IACUC) Approval Number UB-IACUC N° 07/2023.

2.3. Experimental Design

We intended to use female rats just attaining sexual maturity and who had a naturally regular estrous cycle, such that any anestrous female should result from chronic exposure to Abamectin.

2.3.1. Screening of Females

A total of about 40 sexually mature female albino rats were used. The animals were acclimatized for a period of 7 days in the laboratory. Each female was monitored for 4 consecutive cycles (20 days) during which vaginal
smears were collected, prepared and viewed under a light microscope (UNICO 380, USA) at X100 for the presence of the different white blood cell types. Photomicrographs of the smears were taken and recorded. This exercise was aimed at ascertaining the regularity of their oestrous cycle before the experiment.

Vaginal fluid from each was taken at approximately the same time during the entire course of the experiment between 7 am and 8 am daily. The vaginal fluid was collected as follows: the animal was held in a supine position with one hand and with the other hand, using a rubber pipette, 0.5 ml (Rabadia et al., 2022) of 0.9% saline was introduced into the vaginal orifice of the animal ensuring the fluid sent into the vagina returns back with pressure. The fluid was sent three to four times ensuring the colour of the fluid had turned from colourless to milky before collecting. The rubber pipette was then rinsed thoroughly using distilled water to remove any residual cells before the experiment.

The fluid was sent into the vagina orifice of the animal ensuring the fluid sent into the vagina returns back with pressure. The fluid was sent three to four times ensuring the colour of the fluid had turned from colourless to milky before collecting. The rubber pipette was then rinsed thoroughly using distilled water to remove any residual cells before the pipette was used for the next rat (Ngadjui et al., 2015). The smear was prepared by carefully placing a drop of the vaginal fluid on the clean microscope slide and spreading with the aid of the rubber pipette used in collecting the fluid (Okafor et al., 2021; Ngadjui et al., 2013). It was allowed to air dry for one hour, then fixed with methanol and allowed to dry for 10 minutes after which it was stained with 3% methylene blue which was also allowed to air dry for 15 minutes. The smears were then examined under the light microscope at X100 Magnification to identify the phase of the oestrous cycle based on the leukocytes present. Then, photomicrographs of the smears were taken and recorded. Only animals with regular cycles were retained and used in the next phase of the experiment.

2.3.2. Induction of Anestrous

Abamectin or Aba (ACARIUS 018 EC) of an initial concentration of 18 g/l, was diluted 100 times by dissolving 0.25 ml of the product into 99.75 ml of distilled water. The choice of distilled water was based on the fact that the product was readily soluble in it and also that the same solvent was used to prepare the administrative doses of the plant extract as well as other substances administered to the animals. Thirty females with regular cycles obtained from the previous phase of the experiment were administered 2 mg/kg of Abamectin for 45 days with vaginal smears prepared and viewed as described earlier.

This chronic exposure of normal cycled females to Aba resulted in an alteration in transition between the stages of the estrous cycle throughout the treatment period. Hence, anoestrus, a period of reproductive quiescence, was observed. In other words, estrus cyclicity was stopped following treatment with Aba. These anestrous females were then used in the next phase of the experiment.

2.3.3. Partitioning and Treatment of Anestrous Females

Thirty female rats with Aba-disrupted cycles were then partitioned into 5 groups of 5 rats each and subjected to treatment with the aqueous extract (D. laxata), distilled water or the standard drug (Table I) for 15 days during which their cycles were assessed following the procedure described earlier. Partitioning and treatment of animals was done as follows:

- Group 1 = Neutral/Normal control group (normal female rats with a regular cycle and that did not receive any substance)
- Group 2 = Negative control group (oestrous cycle-disrupted female rats that received distilled water)
- Groups 3–5 = Test groups (oestrous cycle-disrupted females treated with dose 1, dose 2 and dose 3 of the plant extract, respectively)
- Group 6 = Positive control group (oestrous cycle-disrupted female rats treated with UTROGESTAN 200 mg)

2.4. Animal Termination, Blood and Selected Organ Collection

After the last (60th) day of the experiment, the animals were starved for a period of 12 hours overnight and sacrificed the following day by cervical dislocation. Reproductive organs including the ovaries, uterus and cervix were collected. They were freed of all connective tissue, rinsed with distilled water, weighed using an electronic scale and morphologically examined. The relative weight of each organ was determined. Also, blood was collected through the jugular vein and introduced into Lithium Heparin test tubes and allowed for 12 hours following which the supernatant was removed, put into clean test tubes and centrifuged for 15 minutes at 3000 rounds/minutes to obtain the plasma which was used for biochemical assay of progesterone and estradiol. Also, a homogenate of the uterus of each rat was prepared by crushing it using a mortar and pestle and 0.1 mL of Tris buffer was added to it. It was transferred into eppendorff tubes which were later centrifuged for 15 minutes at 3000 rounds following the collection of the supernatant. The supernatant collected was also used for biochemical assay. The hormonal assay was done following the protocol described in the assay kit by the manufacturer.

2.5. Statistical Analyses

Values were expressed as Mean ± SEM. Mean values were calculated for each animal and quantitative comparisons between groups were established from those means. Analysis of Variance (ANOVA) followed by Duncan test was used in the SPSS for Windows version 20.0 software. Significant levels were tested at p < 0.05.

<table>
<thead>
<tr>
<th>TABLE I: GROUPING AND TREATMENT OF ANIMALS</th>
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<tbody>
<tr>
<td>Group</td>
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<td>Group 1</td>
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<td>Group 5</td>
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<td>Group 6</td>
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</table>
3. Results

3.1. Effects of the Aqueous Extract of the Leaves of Dicliptera Laxata on the Cyclicity of the Estrous Cycle of Aba-Treated Female Rats

As mentioned earlier, chronic exposure of normal cycled females to Aba resulted in an alteration in transition between the stages of the estrous cycle throughout the treatment period. Hence, anestrus was observed. In other words, estrus cyclicity was stopped following treatment with Aba.

However, assessment of the histology of the vaginal smears of the female rats that received Aba after a 15-day treatment with D. laxata leaf aqueous extract at 75, 150 and 300 mg/kg demonstrated a return to normal cyclicity of four-phased estrous cycle including proestrus, estrus, metestrus and diestrus compared to those treated with distilled water. Animals treated for 15 days showed a constant appearance of either proestrus, estrus, metestru or diestru phase in both those treated with the standard drug and those treated with the plant. Within the second week of treatment, animals treated with the 300 mg/kg body weight dose of the leaf aqueous extract recorded a 100% regulation rate compared to their distilled water and Utrogestan counterparts that registered a 20% and 80% regulation rate, respectively (Table II).

3.2. Effects of the Aqueous Extract of the Leaves of Dicliptera Laxata on the Percentage Weight of Organs of Aba-Treated Female Rats

Administration of the insecticide, Aba caused a significant drop in the percentage weights of ovaries, uterus and cervix in the Aba-treated group compared to the distilled water-treated group. Treatment with Dicliptera laxata leaf-aqueous extract provided a non-significant rise in the relative weights of ovaries, uterus and cervix. Also, Utrogestan provoked a non-significant rise in percentage weight of ovaries, uterus and cervix, compared to the negative control group (Fig. 1).

3.3. Effects of the Aqueous Extract of the Leaves of Dicliptera Laxata on Plasma Estradiol Concentration

Administration of Aba (Acarius 018EC) provoked a significant decrease in the serum concentration of Estradiol in the negative control group (44.90 ± 7.63 pg/mL) compared to the neutral control group (81.38 ± 4.15 pg/mL). Treatment with D. laxata leaf-aqueous extract caused a significant drop in the percentage weight of ovaries, uterus and cervix, compared to the negative control group with 44.90 ± 7.63 pg/mL. Also, Utrogestan significantly increased the uterine concentration of Estradiol with 76.49 ± 5.54 pg/mL compared to the negative control group (44.90 ± 7.63 pg/mL) (Fig. 3).

3.4. Effects of the Aqueous Extract of the Leaves of Dicliptera Laxata on Uterine Progesterone Concentration

Estrous cycle interruption with Aba (Acarius 018EC) induced a significant decrease in the uterine concentration of Progesterone in the negative control group (15.67 ± 2.60 pg/mL) compared to the neutral control group (27.93 ± 0.98 pg/mL). Treatment with D. laxata leaf-aqueous induced a significant (P < 0.01 and P < 0.001) increase in uterine progesterone concentration at 75, 150 and 300 mg/kg with 16.13 ± 0.69, 25.88 ± 1.81 and 27.01 ± 1.22 pg/mL respectively, compared to the negative control group with 15.67 ± 2.60 pg/mL. Also, Utrogestan significantly increased the uterine concentration of Progesterone (27.87 ± 1.37 pg/mL) compared to the negative control group (15.67 ± 2.60 pg/mL) (Fig. 4).

4. Discussion

In the present investigation, we sampled the most widely used pesticide within the Buea municipality, Aba, and assessed the effects of an aqueous extract of leaves of Dicliptera laxata on the Acarius-blocked estrous cycle in female rats to provide a pharmacological justification for the claimed folkloric use of this plant in the treatment of female reproductive inadequacies by the people of Fako Division, South-West Region of the Republic of Cameroon. Analyses of the composition of the plant...
**TABLE II:** Summary of the Evolution of the Estrous Cycle of Aba-Exposed Female Rats Following Treatment with the Aqueous Extract of the Leaves of *Dicliptera laxata*

<table>
<thead>
<tr>
<th>Duration of treatment (Days)</th>
<th>Treatment</th>
<th>Initial number of females</th>
<th>Number with regulated cycle</th>
<th>Percentage (%) regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–7</td>
<td>Neutral</td>
<td>5</td>
<td>5</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Aba + distilled water</td>
<td>5</td>
<td>0</td>
<td>0%</td>
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<tr>
<td></td>
<td>Aba + D.I.75 mg/kg</td>
<td>5</td>
<td>0</td>
<td>0%</td>
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<tr>
<td></td>
<td>Aba + D.I.150 mg/kg</td>
<td>5</td>
<td>1</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>Aba + D.I.300 mg/kg</td>
<td>5</td>
<td>3</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td>Aba + uttrogestan</td>
<td>5</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td>8–15</td>
<td>Neutral</td>
<td>5</td>
<td>5</td>
<td>100%</td>
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<td></td>
<td>Aba + distilled water</td>
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<td>Aba + D.I.75 mg/kg</td>
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<td>Aba + D.I.150 mg/kg</td>
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<td>5</td>
<td>4</td>
<td>80%</td>
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</table>

Abbreviations: Aba: Abamectin (Acarius 018EC); D.I: *Dicliptera laxata*. Neutral = normal female rats with a regular cycle and that did not receive any substance.

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revealed that it contains steroids, phenolic compounds, alkaloids and saponins. As mentioned earlier, insecticides cause a disruption in the female endocrine system and subsequent alterations in reproductive organs as well as germ cells (Litchfield, 2005). Their actions disrupt the physiology of the ovary in two dimensions: alter ovarian functions causing changes in hormonal secretions and these endocrine changes, in turn, affect the female reproductive system via the Hypothalamo-Pituitary-Gonadal axis, resulting in dysfunctions. The main female dysfunctions include disruption in the biosynthesis of hormones and alteration in the maturation of follicles which lead to a disrupted ovarian cycle. Other consequences of insecticide intoxication can be a prolonged gestation period, stillbirth and infertility (Jeyaratnam, 1990). In addition, insecticides could affect germ cells at primordial phases resulting in infertility at the adult stage (Kara & Öztaş, 2020). Negative implications of insecticides in the female reproductive system have been. For example, endosulfan is known to trigger apoptosis by inducing oxidative stress in the follicle cells and also induces the expression of steroidogenic acute regulatory proteins (StAR) CYP19A and aromatase, thus, provoking improper ovarian maturation; dichloro-diphenyl-trichloroethane (DDT) exposure inhibits CYP450-side chain cleavage enzyme, progesterone receptor, estrogen sulfotransferase, cyclooxygenase-2 (COX-2) and epidermal growth factor (epiregulin) which all lead to the alteration of ovulation time (Jeyaratnam, 1990); chlorpyrifos alters uterine weight and morphology by causing the thickness of surface epithelium and myometrium (Bretveld et al., 2006) and also reduces the levels of luteinizing hormone (LH), estrogen and progesterone (Sharma et al., 2020, Ghuman et al., 2013).

In this study, examination of the histology of the vaginal smears of the female albino rats following a 15 days screening period revealed regular cycles with 4 phases: comprising proestrus, estrus, metestrus and diestrus in succession: stages which are characterized by the presence...
of cornified cells, intermediate cells and/or leukocytes found in the vaginal smear (Beck et al., 2008, Weshood, 2008, Byers et al., 2012). Subsequent treatment of these normal cycled female rats with Aba (Acarius 018EC) induced a significant lengthening of the oestrous cycle due to an increase in the length of the anestrus phase and some of the proestrus phase as confirmed by the photomicrograph of vaginal smears of treated rats. This might have been due to the disruption of the ovarian follicles and decreased levels of estrogen produced by Aba (Acarius 018EC)-induced oxidative stress (Soujanya et al., 2022). This insecticide could also have inhibited the release of both LH and FSH via its action on the hypothalamic/pituitary axis, which, in turn, reduced the plasma levels of estradiol and progesterone in Aba (Acarius 018EC)-treated rats and lowered levels of estradiol and progesterone might have disturbed the estrous cycle, and consequently interfered with ovaries and uterus by inducing cytological and biochemical modifications in Aba (Acarius 018EC)-treated rats (Rao & Kaliwal, 2002). Treatment with Dicliptera laxata leaf-aqueous extract provided a dose-dependent 20% and 100% return to normal cyclicity of the estrous cycle in females treated with the 150 mg/kg and 300 mg/kg doses, respectively. This could be explained by the regeneration of ovarian follicles due to the gonadotropic function of the plant extract that resulted in increased production of estrogen by ovaries and led to regularization of the estrous cycle (Soujanya et al., 2022). In this study, oestrous cycle disruption induced by oral administration of Acarius 018EC caused all rats that received distilled water and those
that received the test substance to record significant drops in the relative weights of ovaries, uterus and cervix compared to the neutral control group. This drop in the relative weight of organs was also obtained in studies carried out by Rao and Kaliwal (2002), Sreelakshmi and Kaliwal (2007), and Nguyen et al. (2022). The decrease in weight of ovaries accompanied by anestrus was probably due to alteration in reproductive hormone synthesis. As reported by Rao and Kaliwal (2002), a decrease in the weight and size of ovaries may also be due to extensive fibrosis and atretic follicles (Sreelakshmi & Kaliwal, 2007). On their part, the reduction in the weight of the uteri might have resulted from the fact that the animals were in diestrus at the time of death and also showed prolonged diestrus (Sreelakshmi & Kaliwal, 2007). Treatment with Dicliptera laxata leaf-aqueous extract provided a non-significant increase in the relative weights of ovaries, uterus and cervix. This increase could be explained by the plant extract’s ability to induce the synthesis of reproductive hormones and inhibit the effects of the pesticide molecules at the receptor sites of hormone-producing and receptive cells. This implies that when the uteri were exposed to D. laxata leaf-aqueous extract in vivo, the extract probably contributed to inducing some modifications in the uterine endometrium that transformed it from a non-receptive to a receptive phase. This in turn would have allowed the implantation and development of the blastocyst if these females were mated with fertile males and followed up to term, hence, its importance in stabilizing risky pregnancies in women. Also, in this study, oestrous cycle disruption induced by Aha administration caused a significant decrease in the serum and uterine concentrations (Sreelakshmi & Kaliwal, 2007) of Progesterone and Estradiol in all experimental
animals compared to the neutral group. A similar result was also obtained by Nguyen et al. (2022). Treatment with Dicliptera laxata leaf-aqueous extract led to a significant increase in the serum and uterine concentration of Progesterone and Estradiol. This might have been due to the estrogenic property of the plant which either activated the receptors for Estrogen or increased the synthesis of Estrogen by improving the secretion of LH and/or FSH (Nguyen et al., 2022).

5. Conclusion

Our findings revealed that prolonged exposure of sexually normal-cycled female rats to Acarius 018 EC resulted in disruption of the estrous cycle (anestrus), a decrease in the relative weight of reproductive organs as well as a significant drop in serum concentration of estradiol and progesterone. However, treatment of the anestrous female rats with the leaf-aqueous extract of D. laxata resulted in a return to the normal cyclicity of the estrous cycle, an increase in the relative weight of reproductive organs and serum hormonal profile. These actions of the plant extract could be attributed to the presence of the secondary metabolites in the extract and this justifies its folkloric use in treating irregular menstruation and stabilizing risky pregnancies in women.

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Conflict of Interest

Authors declare that they do not have any conflict of interest.

References


