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The Deleterious Effect of Orally Administered Aqueous Extract of Acalypha *Wilkesiana* (Red Acalypha) on Female Reproductive Organs in Sprague Dawley Rats

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Abstract

Acalypha wilkesiana is a herbaceous plant that has been traditionally used to treat and/or manage a variety of illnesses, including respiratory issues, diabetes, jaundice, hypertension, fever, diarrhea, and inflammation of the liver. The effects of taking Acalypha wilkesiana orally on the uterus and ovaries were examined in this study. Twenty (20) adult female Sprague-Dawley rats (165±20g) were purchased and given a two-week period to get used to the laboratory environment. Aqueous extract of Acalypha wilkesiana was given orally for four weeks at concentrations of 0, 100, 200, and 400 mg/kg of BW following acclimatization. Groups A-D provided as the control, low, medium, and high doses, respectively. The rats' weight gain and behavioral patterns were periodically examined. Following their euthanasia, blood was drawn via the ocular sinuses to measure hormonal parameters, and the uterus and ovaries were exercised to examine histology and oxidative stress markers. Significantly lower levels of FSH, LH, estrogen, and progesterone were observed in the treatment groups, while significantly lower levels of SOD and CAT and higher levels of MDA were observed. In both the 40 and 80 mg/kg groups, histological sections of the ovaries and uterus revealed vascular congestion in the ovaries and moderate to severe damage within the uteri. The findings demonstrated that long-term use of Acalypha

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wilkesiana increased levels of oxidative stress, altered hormone levels, and negatively affected the histoarchitecture of the uterus and ovaries.

Keywords: *Acalypha wilkesiana*, pregnancy, estrous cycle, fetal parameters, uterus, ovary

INTRODUCTION

Though it grows worldwide, *Acalypha wilkesiana* is most commonly found in the tropics of Asia, America, and Africa. Whereas ornamental species may have been brought to West Africa from other parts of the world and are grown as foliage plants in gardens and greenhouses, weeds are ubiquitous and grow wild (Ikewuchi *et al.,* 2010). According to Shin and WHO (2009), the plant has a maximum height of two to four meters and can occasionally reach six meters. The stem is upright and has numerous branches, each with a tightly packed crown and fine hairs. The leaves are broadly ovate to elliptic in shape, up to 20 cm long, 10 to 15 cm wide, and 5 to 7 nerved from the base. They have a serrated or coarsely toothed margin. They are mottled in various shades and patterns of purple, red, pink, yellow, cream, or white, and they can be green, copper, or bronze. At the tips of branches, there are spikes of ten to twenty reddish flowers. According to Elkhouly *et al.* (2017), the fruits are tiny, three-lobed capsules that hold many hard, tiny seeds. Studies have shown that the plant Acalypha wilkesiana has been traditionally used to treat and/or manage a wide range of illnesses, including diabetes, jaundice, hypertension, fever, liver inflammation, schistosomiasis, dysentery, respiratory issues like bronchitis, asthma, and pneumonia, as well as skin conditions like scabies, eczema, and mycoses (Seebaluck *et al.,* 2015). West Nigerians treat infant fungal infections with a boiling decoction of leaves. Ikewuchi *et al.* (2011) reported that the leaves of this plant possess anti-inflammatory, anti-microbial, and anti-pyretic properties. Although there have been reports of *Acalypha wilkesiana*, medicinal plants should be safe. A. wilkesiana has a number of potentially lethal side effects, including direct negative effects, allergic reactions, contaminant effects, and interactions with other medications and herbal remedies. Because they are "natural," phyto-therapeutic products are frequently mistaken to be less toxic. However, these products include bioactive ingredients that might have negative effects. Certain herbal medicines have inherent toxicity that can cause kidney damage. Herbs with unknown toxicity can be harmful or exacerbate the nephrotoxic effects of conventional therapy; preparations tainted with harmful non-herbal compounds may cause a harmless herb to be mistaken for a toxic one; or an incorrect diagnosis may result in the ingestion of a toxic herb. The purpose of this study is to examine the possible toxicity of females' aqueous leaf extract of the purportedly therapeutic plant Acalypha wilkesiana in relation to the effect on the uterus and ovaries (Idris *et al.,* 2020).

MATERIALS AND METHODS

Study Area

A total of twenty (20) adult female Sprague-Dawley rats, weighing 165±20g, were obtained from Bowen University's Physiology Department in Nigeria. The research was carried out in the Anatomy Department's animal house, they were housed in industrial cages at room temperature and were given two weeks to get used to their new surroundings. During the experiment, they were given water and rat chow.

Collection of Samples

Leaves of *Acalypha wilkesiana* were collected from the Bowen University campus in Iwo, Nigeria, and examined by a botanist for identification. No chemicals were used in the air-drying process of the leaves. **Procedure for obtaining samples**

Using an electrically powered blender, the dried leaves were ground into a powder. Of the powdered sample, about 92.4g were obtained. Each of the four groups, A through D, comprising five (5) female rats each, was randomly assigned to the twenty rats. During a 4-week period, the animals were given free access to rat chaw and water, and Group A, which served as the control, received 0 mg/kg of the extract, Group B, which served as the low dose, Group C, which served as the medium dose, and Group D, which served as the high dose. 24 hours after the last dosage, the animals were put to death. The organs' histoarchitecture was determined by fixing the left ovaries and half of the uteri in Bouin's fluid. The levels of oxidative stress were measured by storing the remaining uteri and the right ovaries in sample bottles at -20°C. Blood for hormonal function tests was taken from the ocular sinus. A standard protocol was followed to process the ovarian and uterine tissues for microscopic examination, and 5µm thick paraffin sections were created. Using Olympus and Leica microscopes, photomicrographs were taken at a magnification of 400 and slides were stained with standard hematoxylin and eosin stains.

Measurement of LH, FSH, Progesterone and Estrogen

The samples were kept at a temperature of -20^{o}Cdegrees Celsius until the analysis day. Using enzyme-linked immunosorbent assay (ELISA) kits (Diagnosis Systems Laboratories, Wester, TX), the serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen, and progesterone were measured in female rats across all groups in accordance with the manufacturer's instructions.

Uterine and Ovarian Homogenate for Antioxidant Activities

Following an ice-cold 1.15% KCl solution wash, the ovaries and uterus were blotted, weighed and homogenized using 0.1 M phosphate buffer (pH 7.2). After adding laboratory sand to a mortar, the tissues were set inside. This was broken up using a pestle. For fifteen minutes, the resultant homogenate was centrifuged at 2500 rpm. Before being analyzed, the supernatant was decanted and kept at -20°C. Superoxide Dismutase (SOD) was tested for its capacity to prevent adrenaline from auto-oxidizing by measuring the rise in absorbance at 480 nm. By monitoring the change in absorbance at 480 nm for five minutes, the enzyme activity was determined. Calorimetric assaying at 620 nm was used to measure catalase (CAT), which was reported as µmoles of H_2O_2 Consumed/min/mg/protein. The technique from a prior study was used to measure malondialdehyde (MDA), an indicator of lipid peroxidation. After the supernatant was discarded, the absorbance at 532 nm was measured. The MDATBA- complex molar extinction coefficient of 1.56 × 105 M-1cm-1 was used to calculate MDA.

Statistics

Using Graph Pad software version 9.5, the data collected from each group were combined and subjected to statistical analysis using ONE WAY-ANOVA and Post Hoc Tukey's tests. The data results were presented as mean ± SEM (standard error of mean), with a significance threshold of $p<0.05$.

RESULTS

No mortality was observed during the process of the experiment. Also, no gross abnormalities of the limb, tail or head were observed in the adult rats throughout the duration of the study.

Effect of orally administered *acalypha wilkesiana* on the body weight of the female sprague dawley rats

There was a significant increase in the body weight of both control and treatment groups (Table 1).

TABLE 1: effect of orally administered *acalypha wilkesiana* on the body weight of the female sprague dawley rats

Group	Before administration	After administration	% weight difference
Control	144.50 ± 2.48	206.00 ± 3.76*	41.10
Low dose	161.50 ± 1.05	212.50 ± 4.49*	31.58
Medium dose	174.50 ± 2.87	237.50 ± 3.83*	36.10
High dose	184.50 ± 3.33	280.00 ± 4.61*	51.76

Values are mean ± standard error of mean; n=5, *p<0.05

Effect of orally administered *acalypha wilkesiana* on the organ weight of ovaries and uteri of female sprague dawley rats

There was a decrease in the weight of both ovaries and uteri with significant in uterine weight when treatment groups were compared to control, similar decrease was recorded when medium and high doses were compared to low dose and when high dose was compared to medium dose (Table 2).

TABLE 2: effect of orally administered *acalypha wilkesiana* on the organ weight of ovary of female sprague dawley rats

Group	Ovaries (g)	Uteri (g)
Control	0.15 ± 0.01	5.20 ± 0.01
Low dose	0.13 ± 0.01	4.67 ± 0.02^{a}
Medium dose	0.12 ± 0.01	3.41 ± 0.02^{ab}
High dose	0.09 ± 0.02	3.11 ± 0.20^{abc}

Values are expressed as Mean ± Standard Error of Mean (SEM). ^{a}p<0.05 significant compared to control; ^{b}p<0.05 significant compared with low dose; ^{c}p<0.05 significant compared with medium dose.

Effect of orally administered *acalypha wilkesiana* on the hormones of female sprague dawley rats

In the values of FSH, LH and Progesterone a dose-dependent decrease was recorded when treatment groups were compared to control, similar decrease was recorded when medium and high doses were compared to low dose and when high dose was compared to medium dose (Table 3).

TABLE 3: effect of orally administered *acalypha wilkesiana* on the hormones of female sprague dawley rats

Group	Fsh	Lh	Progesterone
Control	2.55 ±0.88	15.54 ± 2.09	65.53 ± 2.31
Low dose	2.21 ± 0.65	12.30 ± 0.60	60.88 ± 1.92^{a}
Medium dose	1.52 ± 0.82^{ab}	11.06 ± 0.57^{a}	47.64 ± 1.29^{ab}
High dose	1.05 ± 0.91^{abc}	7.24 ± 0.14^{abc}	35.62 ± 0.48^{abc}

Values are expressed as Mean ± Standard Error of Mean (SEM). ^{a}p<0.05 significant compared to control; ^{b}p<0.05 significant compared with low dose; ^{c}p<0.05 significant compared with medium dose.

Effect of orally administered *acalypha wilkesiana* on the oxidative stress levels of the ovaries and uteri female sprague dawley rats

In the values of the MDA, a dose dependent increase was recorded when treatment groups were compared to control and decrease was seen in the SOD and CAT values when treatment groups were compared to control (Table 4).

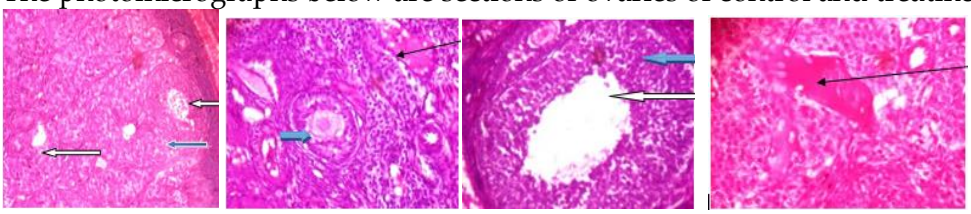
TABLE 4: effect of orally administered *acalypha wilkesiana* on the oxidative stress levels of the ovaries and uteri in female sprague dawley rats

Ovary			
Group	Malondialdehyde	Superoxide dismutase	Catalase
Control	306.42 ± 2.77	430.31 ± 8.09	388.06 ± 3.93
Low dose	370.90 ± 8.44[a]	399.67 ± 2.02[a]	348.51 ± 1.89[a]
Mediumdose	445.58 ± 8.96[ab]	333.20 ± 2.93[ab]	305.55 ± 2.93[ab]
High dose	521.98 ± 8.49[abc]	295.01 ± 1.09[abc]	259.37 ± 0.63[abc]
Uterus			
Control	290.81 ± 3.69	401.37 ± 5.81	392.94 ± 1.33
Low dose	319.96 ± 2.88a	364.11 ± 5.05a	366.39 ± 0.92a
Medium dose	399.48 ± 6.83ab	302.07 ± 3.11ab	289.07 ± 2.77ab
High dose	451.40 ± 8.94abc	288.09 ± 4.72abc	244.60 ± 1.57abc

Values are expressed as Mean ± Standard Error of Mean (SEM). [a]p<0.05 significant compared to control; [b]p<0.05 significant compared with low dose; [c]p<0.05 significant compared with medium dose.

EFFECT OF ORALLY ADMINISTERED *ACALYPHA WILKESIANA* ON THE OVARIAN CYTOARCHITECTURE FEMALE SPRAGUE DAWLEY RATS

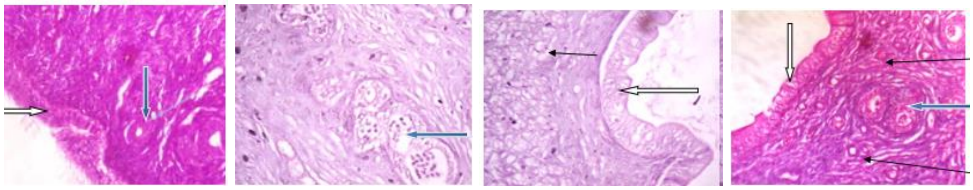
The photomicrographs below are sections of ovaries of control and treatment groups



Control Low dose Medium dose High dose

Section of Control showing some normal antral follicle (white arrow) with normal theca cells (blue arrow) within the ovarian cortex, the ovarian stroma show normal connective tissues and luteinized stromal cells; Section of low dose showing ovarian stroma with mild vascular congestion (slender arrow) and Luteinized stroma cells; Section of medium dose showing stroma with mild to moderate vascular congestion; Section of high dose showing stroma with moderate vascular congestion (slender arrow).

EFFECT OF ORALLY ADMINISTERED *ACALYPHA WILKESIANA* ON THE UTERINE CYTOARCHITECTURE FEMALE SPRAGUE DAWLEY RATS



Control Low dose Medium dose High dose

The photomicrographs below are sections of uteri of control and treatment groups

Section of Control showing normal endometrium epithelial layer (white arrow), there are normal endometrial gland (blue arrow); section of low dose showing mild proliferation of endometrial gland (blue arrow) with sloughed epithelial cells and there is moderate infiltration of the endometrial stroma by inflammatory cells; section of medium dose showing mild proliferation of endometrial gland seen with sloughed epithelial cells, there is moderate infiltration of the endometrial stroma by inflammatory cells (slender arrow); section of high dose showing moderate proliferation of endometrial gland seen (blue arrow) and there is

severe infiltration of moderately fibrotic endometrial stroma by inflammatory cells (slender arrow).

DISCUSSSION

Rats that were given Red Acalypha experienced an increase in body weight following administration; however, the rate of increase was contingent upon the dosage of grounded Red Acalypha; the higher the dose of Red Acalypha, the slower the rate of weight gain. By comparing the treatment groups with the control rats, this ratio was determined. This is due to the fact that, in comparison to the treatment groups, the control rats' body weights increased significantly. It's likely that this is the result of receiving no Bay leaf medication. This means that Red Acalypha may not reduce body weight during the course of the recommended 19 days of administration, but over time, weight reduction may occur if the medication is taken for a longer period of time and at a higher dose.

Comparing the uterine weights of the treatment groups to the control group revealed a decrease, but not in a dose-dependent way. The uterine weight of the low dose group was the lowest, followed by that of the medium dose group and the high dose group. The difference between the uterine weight of the high dose group and the control group was not very great. Comparing the ovarian weights of the other treatment groups and the control group, the medium group had the highest weight.

When comparing the treatment groups' serum levels to those of the control group, it was observed that the three reproductive hormones under analysis had decreased in a dose-dependent manner. According to Orlowski and Sarao (2021) follicle stimulating hormone is the hormone that grows ovarian follicles within the ovary prior to their release as developed eggs during ovulation. Luteinizing hormone regulates the menstrual/oestrous cycle and is the hormone that causes the ovary to release an ovum (Casarini *et al.,* 2018). Progesterone is a sexual hormone secreted by the corpus luteum that helps the body get ready for pregnancy (Furth *et al.,* 2018). The levels of progesterone, luteinizing hormone, and follicle stimulating hormone decreased in all treatment groups in a dose-dependent manner, with the low dose rats exhibiting the highest levels of these three reproductive hormones and the high dose rats the lowest. Malondialdehyde (MDA), a production of polyunsaturated fatty acids peroxidation in the cells, and a toxic molecule were found in the oxidative stress analysis result of the groups' ovaries and uteruses (Gaweł *et al.,* 2022). In the treatment groups, it rose in a dose-dependent manner, with the high dose rats having the highest level and the low dose rats having the lowest. The SOD levels of the high dose rats were lowest and those of the low dose rats were highest, suggesting that the high dose rats' cells may contain elevated concentrations of potentially hazardous oxygen. All aerobic organisms contain the antioxidant enzyme catalase (CAT). In cells under environmental stress, it is known to catalyze the energy-efficient conversion of hydrogen peroxide (H_2O_2) into water and oxygen (Sharma and Ahmad, 2014). It is also crucial for shielding the cell from reactive oxygen species-caused oxidative damage. When compared to the control group, the CAT levels in the uteruses and ovaries decreased in the treatment groups in a dose-dependent manner. Within the treatment group, the low dose rats exhibited the highest level of catalase, whereas the high dose rats displayed the lowest level. The rats in the treatment group administered with a high dose of Bay leaf had significant effects on their uteri and ovaries. The ovarian stroma of the high dose rats exhibits mild vascular congestion, but the endometrial stroma of the uterus displays severe infiltration by inflammatory cells.

CONCLUSION

The findings of this study indicate that Acalypha wilkesiana may have a negative impact on the release of FSH, LH, estrogen, and progesterone in pregnant Sprague Dawley rats, as well as cause damage to the uterus and ovaries.

RECOMMENDATION

The authors recommend that caution should be applied with the consumption of this extract as it could possibly impact the changes of fecundity as this study has demonstrated the damages on the female reproductive system.

Conflict of Interest

The authors declare no conflict of interest.

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