EFFECT OF CORDIA MILLENII EXTRACT ON REPRODUCTIVE HORMONE IN CISPLATIN INDUCED INFERTILITY IN FEMALE ALBINO RATS

Nnadozie, Ijeoma Jane1, Olajide, Omolola Caroline2, Agbabiaje, Olayinka Oluwasegun3, Okpuzor, Joy4

1-4Department of Cell Biology and Genetics, University of Lagos, Akoka – Lagos, Nigeria

Correspondence Author:
Nnadozie, Ijeoma Jane
Email: ijeomajannnadozie@yahoo.com

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Abstract
Cordia millenii is a plant that belongs to the family Boraginaceae family, commonly known as African Cordia. In Nigeria, its Igbo name is okwe, omo in Yoruba and waawankurmii in Hausa. Various parts of Cordia millenii has been used as a restorative medicine for treatment of fever, general body weakness and stomach- ache. The present study was carried out to determine the effect of graded doses of ethanol leaf extract of Cordia millenii on fertility hormone in cisplatin induced infertility of female albino rats. Twenty four (24) healthy female rats weighing between 95 kg – 140 kg were divided into five groups. Group 1 served as the control, the animals in group 2 received an intraperitoneal dose of cisplatin (5mgkg⁻¹ body weight). Animals in groups three four and received combination of oral dose of Cordia millenii ethanol extract 100 mgkg⁻¹, 200 mg kg⁻¹ body weight and cisplatin in 5 mg kg⁻¹, while animals in group five received an oral dose of Cordia millenii extract only (100 mgkg⁻¹) for 14days. At the end of the study two rats were randomly selected from the cisplatin group for the mating study and blood samples were taken from the other rats in various groups for hormonal analysis. The result from the mating study revealed that cisplatin caused an irreversible infertility in the rats as no conception was achieved after 8 weeks. The estrogen level reduced in the cisplatin, low and high dose of Cordia millenii plus cisplatin and Cordia millenii extract only groups. Results from this study have shown the antifertility effect of Cordia millenii ethanol extract. Therefore, awareness should be created to avoid using Cordia millenii leaves in the treatment of infertility as currently; traditional health practitioners believe that this plant enhances fertility.

Keywords: Infertility, Cisplatin, hormone, ethanol extract, Albino rats.

1. INTRODUCTION
Infertility is one of the most important and common medical conditions of the reproductive system. It is defined as inability of couples to conceive pregnancy after 12 months of regular, unprotected intercourse [1]. Female infertility occurs in about 37% of all infertile couples and thus accounts for 35-40 % of overall infertility [2]. In addition, infertilities, either primary or secondary will occur for almost 15% of all women worldwide [3]. In Nigeria, surveys have shown that the prevalence of infertility has continued to increase and currently affects 30% or more of the women [4]. The most common causes of female infertility are hormonal. These are commonly associated with ovulation, ovarian syndrome, pre - mature ovarian failure, damage to the fallopian tube or uterus and problem with cervix. Endocrine disorders result from excessive production of hormones or insufficient production of one or more hormones or the lack of the tissues responses to normal circulating hormones [5]. The female reproductive cycle function primarily by the interplay between the luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone, estradiol and prolactin. The integrity of the female reproductive organs could be assessed by the assaying the serum level of these hormones [6].

In Nigeria, medicinal plants are widely known to be used by herbal practitioners in the treatment of several medical conditions such mental illness, insomnia, malaria and infertility etc [7]. The therapeutic and pharmacological importance of these medicinal plants is found in its non-nutrient bioactive compounds which are often referred to as phytochemical components. [8]. It has been reported that 577 plant species have been used traditionally in fertility regulation in females. [9]. Medicinal plants such as Cissampelos pareira, Hibiscus


rosa-senensis, and Indigofera linnae have been demonstrated to possess antifertility activities [10; 11; 12]. However, very few documented cases of pro-fertility plants are seen in literatures.

*Cordia millenii* (Boraginaceae) is commonly known as African *Cordia*. The plant has been reported to possess numerous biological activities [13]. The seeds of the plants have been used in the treatment of ringworm and itching skin. Other parts of the plant are used in the treatment of asthma, cough and cold. Previous phytochemical studies done on *C. millenii* reported the presence of phenolic compounds, triterpenoids, Cardiac glycoside, alkaloids, flavonoids and steroids [13; 14]. The leaves of *C. millenii* have been reportedly used by traditional medicine practitioners as a fertility enhancer in females among the Yoruba’s in south western part of Nigeria. However, to date, there has not been any scientific report to substantiate this claim that the leaves of *Cordia millenii* have fertility enhancing properties. So the present investigation is directed at evaluating the effect of *Cordia milleni*ethanol extract on female reproductive hormone using albino rats’ models.

2. MATERIAL AND METHODS

2.1: Preparation of the extract

The leaves of *Cordia millenii* were purchased from a traditional herbal practitioner in Mushin market, in Lagos Metropolis. The leaves of *C. millenii* were air-dried for two weeks, blended to a fine powder and soaked in 1.5L of 70% ethanol for 72 hours after which it was filtered using muslin cloth. Thereafter, the filtrate was freeze-dried and the yield was 15.3g of crude extract. A voucher specimen with number LUH7608 for the plant was deposited in the herbarium of the Department of Botany, Faculty of Science, University of Lagos.

2.2: Phytochemical screening

Phytochemical screenings for alkaloids, tannins, saponins, flavonoids, cardiac glycosides, phlobatannin and steroids were carried out on the concentrated extract, according to the methods of [15;16].

2.3: Experimental Animals

Twenty four female wistar rats and 16 female albino mice weighing 95- 140kg and 17- 23kg respectively were used for this research, the animals were purchased from Mr. Jide’s animal farm house located in Abeokuta, Ogun State, Nigeria. The rats were divided into five groups; the treatments for each group were as follows: Group 1 which served as control contained five rats, group 2 had six rats while groups 3 to 5 had four rats each. Induction of infertility was performed by intraperitoneally injecting cisplatin at a dosage 5mgkg⁻¹ body weight of the animals for 14 days in 1ml daily of extract at 24 hours interval in between them [17]. Rats in group 2 were administered an intraperitoneal dose of Cisplatin at a dose of 5 mgkg⁻¹ body weight. Groups 3 and 4 rats received oral dose of *Cordia millenii* extract at 100 mgkg⁻¹ body weight, 200 mgkg⁻¹ body weight and Cisplatin at (5 mgkg⁻¹ i.p) while animals in group 5 received only *Cordia millenii* extract 100 mgkg⁻¹ body weight. The treatment lasted for fourteen days.

2.4: Acute Oral Toxicity of the Plant Extract

The acute toxicity study was performed to determine the safe dose of *Cordia millenii* ethanol extract before administration to rats. Sixteen adult female albino mice (17- 23g) were divided into four groups with four mice in each group and fasted overnight. Graded doses of the plant extract were administered orally, ranging from 100,200, 400 and 1000 mgkg⁻¹ bodyweight, respectively to different groups of mice. The animals were observed for 72h for behavioural changes and mortality.

2.5: Experimental Procedure

Blood samples were obtained from the rats in each group by ocular puncture into plain bottles and EDTA bottles. Estradiol analyses were performed on the serum samples obtained from the animals using commercial standard Enzyme – Linked Immunosorbent Assay (ELISA) kit. The ovaries tissues were carefully harvested for histology procedure as described by [18].

2.6: Mating Study

Mating study was carried out to determine the level of cisplatin damage on the female reproductive system. Two female rats were treated with cisplatin for 10 days and then mated with a healthy male rat for 28 days (4 weeks).

2.7: Statistical Analysis

Statistical significance of difference in two variables i.e. treated group and control groups were evaluated by using one way analysis of variance (ANOVA, SPSS Version 23) and expressed as mean ± Standard Error Mean (SEM). Differences between groups were regarded significantly at P<0.05.

3. RESULT

3.1: Preliminary phytochemicals screening

Qualitative phytochemical analysis of the extract revealed the presence of phenol, tannin, terpenoid, phlobatansins, saponin, alkaloids, flavonoids, steroids, and cardiac glycoside, however, only reducing sugar were not detected (Table 1)

Table 1: Qualitative phytochemical Screening of ethanol extracts of *Cordia millenii* leaves...
### Table 1: Phytochemical Tests and Observations

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemicals</th>
<th>Tests</th>
<th>Observations</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phenol</td>
<td>Ferric chloride test</td>
<td>Dark green coloration</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Tannin</td>
<td>Ferric chloride test</td>
<td>Blue-black precipitation</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Terpenoid</td>
<td>Chloroform+H₂SO₄</td>
<td>Reddish-brown coloration</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Phlobatanins</td>
<td>%1HCL</td>
<td>Red Precipitation</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponin</td>
<td>Froth test</td>
<td>Persistence of frothing upon vigorous shaking</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Alkaloids</td>
<td>Dragendorff’s reagents</td>
<td>Blue-black precipitation</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoid</td>
<td>NH₃+H₂SO₄</td>
<td>Yellow coloration</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Steroid</td>
<td>Libermann Burchard’s test</td>
<td>Violet to blue or green</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Reducing Sugar</td>
<td>Fehling’s test</td>
<td>Absence of Red Precipitation</td>
<td>−</td>
</tr>
<tr>
<td>10</td>
<td>Cardiac glycoside</td>
<td>Keller-Kiliani test</td>
<td>Greenish ring above brown ring</td>
<td>+</td>
</tr>
</tbody>
</table>

#### 3.2: Effect of *Cordia millenii* ethanol extract on plasma estrogen levels

Oral administration of ethanol extract of *C. millenii* to rats caused a significant decrease (p<0.05) in estrogen levels when compared to the control group as shown in (Figure 1).

![Graph](http://www.jabsdirect.cf)

**Figure 1:** Effect of ethanol leave extract of *C. millenii* on female rat estrogen level

* Statistically significant, P<0.05 compared with the control
3.3: Effect of Ethanol Leaves Extract of *Cordia millenii* on the Ovaries of Female Rats

The photomicrographs (Plates A-E) as revealed from histology reports of the ovaries of the rats show that the cisplatin treated groups had increased regressing corpus luteum, few distorted primordial and primary follicles. Atretic follicles and distorted granulosa cells were also seen.

**Figure 2:** Plates A-E: Cross section of the ovary of control, Cisplatin and Cordia millenii extract treated female rats at doses of 100 and 200 mg kg⁻¹ body weights for 14 days. Primordial Follicle – PM, Primary Follicle – PF, Secondary Follicle – SF, Granulosa Epithelium- GE, Antrum- A, Oocyte- O, Granulosa Cell- G, Regressing Corpus Luteum- RCL, Atretic Follicle- AF, Large Mature Follicle- LMF
3.4: Mating Study
The mating study revealed that none of the female rats were able to achieve conception for the time duration of 28 days.

4. DISCUSSION
The phytochemical investigations of the *C. millenii* extract from our results are in agreement with the results reported by [13; 14]. The *C. millenii* extract is safe for administration to rats because even at a dosage of 1000mgkg⁻¹ body weight no behavioural changes and mortalities were observed after 72hours. Bioactive components present in medicinal plants have been reported to have an effect on the regulation of oestrous cycle, conception and reproduction. [19]. [20] described alkaloids known as bioactive components which is contained in most medicinal plants has been described as an inhibitor of aromatase enzymes, thus altering the potential for steroid production and reproductive hormones performance. Aromatases are essential enzymes required in the conversion of androgen to estrogen needed during the female reproductive cycle. Thus, it is possible that the ethanol extract of *Cordia millenii* contains biological active phytochemicals and plant toxins which may alter the functions of the endocrine system in the female rat models used in this study. The significantly (p < 0.05) reduced plasma estrogen level after oral administration of ethanol extract of *Cordia millenii* in the test groups compared to the control may be a pointer to disruption of the oestrous cycle in rats. Estrogen is one of the active sex hormone known to play a key role in the different stages of ovarian development in females. An optimum balance in the level of estrogen is important in increasing the surge of luteinizing hormone which is required to induce ovulation. After ovulation, at subsequent time the corpus luteum secretes progesterone which promotes implantation and establishment of pregnancy, any alteration in the level of these hormones may lead to irregularities of ovulation, hence reducing the level of progesterone and impeding implantation [21; 22]. The resulting consequence of these is spontaneous abortion and failure of implantation and this could finally result into infertility [22; 23].

Cisplatin is a known chemotherapeutic drug used in the treatment of many solid tumors, mainly testicular and ovarian tumors [17]. Chemotherapeutic drug such as cisplatin has been reported to cause temporary or permanent infertility, ovarian damage of the primordial follicular cells thereby leading to ovarian dysfunction [24]. Results from the mating study carried out on the female rat’s shows their inability to achieve conception after 28days, this could be as a result of the administration of cisplatin which could cause irreversible infertility, and the damaging effect of cisplatin on ovarian follicles has been previously reported by other researchers [24; 25]. However ovarian tissues treated with *Cordia millenii* extract and cisplatin in a low and high dosage administration showed a remarkable recovery of the secondary, primary and primordial follicles architecture and decreased in primary and secondary follicles distortion. The finding from these studies indicate that the ethanol leaf extract of *Cordia millenii* in a graded dosage could have a protective effect as well as diminishing the oxidative stress exacted on the ovary tissues by cisplatin, thus consequently improving ovarian follicles architecture in female rats.

5. CONCLUSION
*Cordia millenii* has shown great therapeutic properties which could be exploited as a pharmacological agent in the prevention and treatment of ovarian toxicity and infertility induced by cisplatin or other chemotherapeutic agents known to cause ovarian dysfunction. However, caution should be taken when administering *Cordia millenii* leaves extract in the treatment of infertility by traditional practitioners as the plant possess anti infertility activities by decreasing the level of plasm estrogen. Further research needs to be undertaking on the possible use of the plant as a potential contraceptive in drug development.

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