# Phytochemical And Abortifacient Effects Of Calotropis procera Leaves In Female Wistar Rats

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#### Abstract

In developing countries, abortions are performed traditionally using plants from the pharmacopoeia, including Calotropis procera. The use of abortive plants in traditional medicineis a practice that often lacks experimental trials thatcould lead to the rational use of plants. In this study, 12 female rats thatwere five days pregnant were selected and divided into two groups. The first group received adose of 2000 mg/kg body weight of the aqueous extract of Calotropis procera, and the second group received distilled water for seven days orally. The phytochemical study of the aqueous extract of Calotropis procera leaves revealed alkaloids, flavonoids, polyphenols, sterols and polyterpenes, saponosides, and catechin tannins. The total aqueous extract of Calotropis procera leaves does not alter the reproductive organs, but influences the maturation of primary and secondary follicles and impacts implantation by reducing the number of embryo attachment sites. A dose of 2000 mg/kg body weight administered to pregnant female rats alters the relative mass of the uterus and reduces the rate of viable fetuses and the number of implantation sites for these fetuses. On the other hand, itincreases the rate of non-viable fetuses and resorption sites. Histopathology revealed no structural changes in the ovaries. **Keywords**: Calotropis procera, female rat, follicles, fetus, abortion

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# I. Introduction

Abortion is the termination of pregnancy. The practice of abortion exists and is a social reality that is regulated to varying degrees. It is carried out in different ways depending on the era, culture, and socio-political context. According to the WHO<sup>1</sup>, approximately 73 million abortions take place each year worldwide. The right to abortion is not universally accepted around the world; it remains illegal in some 20 countries, notably in South America and Honduras. In the United States, where abortion laws are being challenged and changed, this has sparked protests and debates. In Europe, a total ban remains the exception, while many African nations prohibit abortion, including Egypt, Senegal, Gabon, Madagascar, and Mauritania. There are two main types of methods used to perform abortions: modern methods and traditional methods<sup>2</sup>. Traditional abortion methods are also practiced by traditional therapists (naturopaths, healers, diviners, etc.). These methods most often result in failure or incomplete abortion, which can lead to complications for women<sup>3</sup>. Among the techniques used in traditional abortion is the use of plants from the pharmacopoeia. The use of abortive plants in traditional medicine a practice that often lacks experimental testing, which can lead to the irrational use of these plants. This study contributes to knowledge about the abortive effect of Calotropis procera, a plant used in African pharmacopoeia. The objective of this study is to determine the major chemical groups and evaluate the abortive effect of Calotropis procera in female Wistar rats.

#### Materials

Plant material

The plant material consists of Calotropis procera leaves. The leaves are harvested at Felix HOUPHOUËT-BOIGNY University (Abidjan/IVORY COAST) between 6:00 and 7:30 a.m.

#### **Animal material**

The animal material consists of nulliparous female rats (*Rattus norvegicus*) of the Wistar strain (Muridae). They are bred at the vivarium of the Ecole Normale Supérieure (ENS) in Abidjan. These animals are frequently used in research because their genetic material is similar to that of humans. They are also used because of their prolificacy (4 to 12 pups per litter), their cycles (4 to 5 days), and the gestation period, whichis 21 days. The rats are raised at an ambient temperature of around 25°C in bins lined with wood shavings. They are fed a mixture of corn powder, bread, and driedfish, and have free access to water.

#### Methods

Fresh *Calotropis procera* leaves are harvested and dried in the laboratory at a temperature of approximately 25°C for twoweeks. The dried leaves are ground using an IKA A10 Labortechnik (Germany) mill to obtain a powder. The powder obtained is macerated by mixing 50 g in 1.5 liters of distilled water and stirred for 24 hours using a magnetic stirrer (JANKE & KUNTELIKA Labortechnik, Germany). After filtering three times through a clean cloth, three times through cotton wool, and once through Whatman No. 1 filter paper, the filtrate is placed in a Memmert oven at 50°C until a dry extract in flake form is obtained and stored at room temperature in closed glass jars.

### Phytochemical screening

Phytochemical screening is a qualitative study that determines the major chemical groups contained in a plant extract through known reactions or processes. The total aqueous extract of *Calotropis procera* obtained was used to identify the chemical groups contained in *Calotropis procera*.

### **Detection of sterols and polyterpenes**

Sterols and polyterpenes are detected using the LIEBERMANN reaction. To do this, five (5) ml of aqueous extract solution of *Calotropis procera* are evaporated on a sand bath. The residue obtained is dissolved in 1 ml of acetic anhydride while hot. 0.5 ml of concentrated sulfuric acid is added to the mixture. The appearance of a purple or violet ring at the interface, turning blue then green, indicates the presence of sterols and polyterpenes.

### **Detection of polyphenols.**

The reaction to ferric chloride (FeCl3) enabled the polyphenols to becharacterized. To do this, a drop of alcoholic ferric chloride solution (2%) was added to 2 ml of *Calotropis procera* solution. The appearance of a blackish-blue or green coloration of varying intensity indicated the presence of polyphenols.

# **Detection of flavonoids.**

Flavonoids are detected by the "cyanidin" reaction. To do this, two (2) ml of *Calotropis procera* solution are evaporated and the residue is taken up in five (5) ml of hydrochloric alcohol diluted twice. The addition of 2 to 3 magnesium chips causes a purplish-pink coloration characteristic of the presence of flavonoids. The addition of 3 drops of isoamyl alcohol intensifies this coloration, confirming the presence of flavonoids.

### **Detection of Tannins**

Tannins consist of catechin tannins and gallic tannins. Catechin tannins are detected using STIASNY' sreagent. Five (5) ml of Calotropis procera solution are evaporated to dryness. After adding 15 ml of STIASNY' sreagent to the residue, the mixture iskept in a water bath at 80°C for 30 minutes. The appearance of a coarse flocculent precipitate characterizes catechin tannins. For gallic tannins, three drops of FeCl3 are added to the filtrate of 5 ml of *Calotropis procera* solution. The resulting solution is saturated with sodium acetate. The appearance of an intense blue-black color characterizes gallic tannins.

### **Detection of quinone substances**

The BORNTRAEGEN reagent was used to detect quinone substances. To do this, two (2) ml of *Calotropis procera* solution are evaporated to dryness. The residue obtained is triturated in five (5) ml of 1/5 hydrochloric acid. The triturate is pouredinto a test tube and placed in a water bath for 30 minutes. After cooling, 20 ml of chloroform are added. The addition of BORNTRAEGEN reagent causes a red or purple coloration, indicating the presence of quinone substances.

# **Detection of alkaloids**

Alkaloids are detected using DRAGENDORFF and BOUCHARDAT reagents. To do this, six (6) ml of the *Calotropis procera* solution are evaporated to dryness. The residue obtained is taken up in six (6) ml of 60° alcohol. This alcoholic solution is divided into three test tubes. In the first tube, the addition of two drops of DRAGENDORFF'S reagent and the appearance of a precipitate or orange coloration indicates the presence of

alkaloids. In the second tube, the addition of 2 drops of BOUCHARDAT reagent and the appearance of a precipitate or a reddish-brown color confirms the presence of alkaloids.

### **Detection of saponosides**

To test for saponosides, ten (10) ml of *Calotropis procera* solution is poured into a test tube 15 cm long and 15 mm in diameter. The tube is then shaken vigorously for 10 seconds and left to stand for 15 min. The appearance of a persistent foam height of more than 1 cm indicates the presence of saponosides.

# Abortive effect of the total aqueous extract of Calotropis procera leaves

For this test, 12 female rats thatwere five (5) days pregnant were selected and divided into two (2) groups of six (6) pregnant female rats. The first group received 1 ml/100 g body weight of the 2000 mg/kg body weight dose of the total aqueousextract of *Calotropis procera* leaves for seven (7) days. The second group received 1 ml/100 g body weight of distilled water for seven (7) days. The animals were weighed regularly until the end of the treatment. Three (3) days after treatment, i.e., on the 15th day of gestation, the animals were sacrificed after anesthesia, the ovaries and uterine horns were removed and weighed, and the number of implantation sites, the number of resorption sites, and the number of viable and non-viable fetuses were counted and recorded. The uterine horns containing the fetuses and the ovaries were dissected and weighed.

# Body weight gain of pregnant female rats

The body weight gain of each group of animals is determined at the end of the experiment using the following formula:

# Weight of organs weighed

**GMC**Mass of pregnant female rats on day 15 - Mass of pregnant female rats on day 1

The relative weight of the organs removed is determined using the following formula:

#### Viable fetus rate

The viable fetus rate (VFR) was determined using the following formula:

### Non-viable fetus rate

The non-viable fetus rate (NVFR) was calculated using the formula below:

#### Rate of non-resorbed implant sites

The percentage of implant sites (TSI) was determined using the following formula:

### **Resorption site rate**

The resorption site rate (RSR) was calculated using the following formula:

# Histopathology of the ovaries of pregnant rats

The ovaries of pregnant female rats are examined histologically. To do this, several steps are followed.

• Fixation of organs

The purpose of fixing the organsis to keep the cells in a state close to that of living tissue. Fixation causes the organ to harden, whichhelps to keep the various tissue structures in place. This protects the cells from bacterial attack and shrinkage. The organs are immersedin 10% formalin for 48 hours at room temperature.

• Dehydration and clearing

Once removedfrom the formalin, the organs are placedindividually in cassettes and dehydrated in four successive baths of alcohol at increasing degrees (80°, 90°, 96°, and 100°) for one hour and twohours, respectively. Afterdehydration, the organs contained in the cassettes are clarified in three successive baths of toluene, each lasting twohours. Clarification consists of removing all traces of alcohol from the organs and preparing them for impregnation.

Impregnation

The organs are impregnated in two baths of liquidparaffin for two and threehours. This operationiscarried out in an oven (MEMMERT, Germany) at between 58 and 60°C.

# • Paraffinembedding

Paraffinembeddingiscarried out at ambient temperature. The cassettes containing the organs are opened by removing the seal. The organieces are removed and placed in the mold. The moldisthencovered by the cassette, intowhichliquidparaffinispoureduntilitis full. Aftercooling, the moldisremoved, leaving a solidparaffin block in which the organisfixed to the back of the cassette. To facilitateremovalfrom the mold, the blocks are hardened in a freezer.

### • Cutting blocks with a microtome

Using a LEICA model microtome (RM 2125 RTS), sections 3µm thick are produced. The blocks on the back of the cassettes are clamped onto the microtome for cutting, producing paraffinstrips containing organ sections.

# • Mounting and deparaffinization of organs

The strips are placed in a water bath at 40°C and thenmounted on microscope slides. These slides are placed in an oven at 58-60°C for 30 minutes to bedeparaffinized. The organ sections spread on the slides are deparaffinizedagain in three successive toluene baths, each lasting 15 min.

### • Rehydration and staining of organ sections

Rehydrationiscarried out in three successive baths of alcohol at decreasing temperatures (100°C, 90°C, and 80°C) for 5 minutes each. The sections are thenrinsed with distilled water.

The organ sections are placed in a hematoxylin-eosin bath. In this technique, there are two types of dye: hematoxylin (nucleardye) and eosin (cytoplasmicdye). Afterrinsingwithdistilled water, the sections were stained withhematoxylin for 2 to 3 minutes, rinsed withtap water, and then immersed in 3% eosin for 3 to 5 minutes.

#### Dehvdration

Afterstaining, the sections are dehydrated again in three increasing alcohol baths (75%, 95%, and 100%) for five minutes each.

### • Mounting and observation of sections

The sections are mountedbetween the slide and cover slip using a few drops of EUKITT embedding medium. An Olympus CX31 (Philippines) triocularelectron microscope toppedwith a camera (AmScope, MD130) connected to a computer (HP Elitebook Folio 1040 China) equippedwithvideo software issued for observations. The magnifications allowed for the assessment of any tissue abnormalities in the organs.

### Statisticalanalysis

The results of the study of the acute toxicity and abortive activity of *Calotropis procera* are presented as mean  $\pm$  standard deviation. The means of the batches are statistically compared by analysis of variance using the Mann Whitney test. Values of p < 0.05 were considered significant. The software used to perform these various tests is STATISTICA.

# II. Results

Phytochemical composition of the total aqueous extract of Calotropis procera

Phytochemical screening showedthat the total aqueousextract of Calotropis procera containsalkaloids, flavonoids, polyphenols, sterols and polyterpenes, saponosides, and catechin tannins. However, no quinone substances or gallic tannins were detected (Table 1).

 Table 1: Phytochemical composition of the total aqueousextract of Calotropis procera

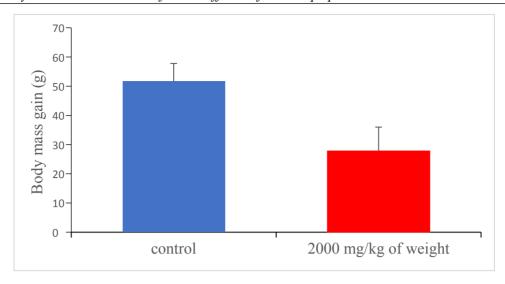
Sterols/ polyterpenes	Polyphenols	Flavonoids	Tannins	Quinones	alkaloids	Saponosides
			Cat Gal			
+	+	+	+ -	_	+	+

Cat= catechins; gal=gallic; +: present; -: absent

# Abortive effect of Calotropis procera on pregnantfemale rats Body mass gains in pregnantfemale rats

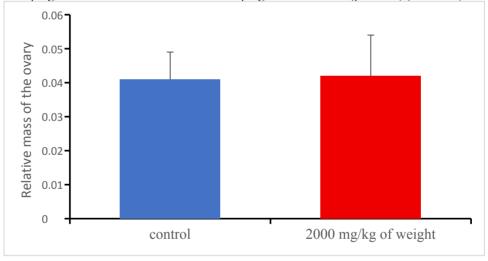
After 15 days of gestation, the average body weight gain of the control female rats was  $51.666 \pm 6.121$  g, whilethat of the treated female rats was  $27.833 \pm 8.158$  g. Statistical analysis shows a significant decrease in body weight gain in treated female rats compared to control female rats (Picture 1).

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# Relative mass of the ovary and uterinehorn of treated rats

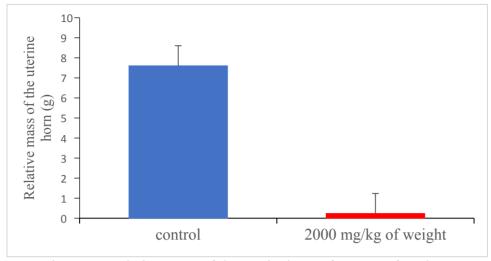
The relative mass of the ovary of treated pregnantfemale rats shows an average of  $0.042 \pm 0.012$  g. The control pregnantfemale rats had an average relative mass of the ovary of  $0.041 \pm 0.008$  g. Statistical comparisons howed that there was no significant difference between the average relative mass of the ovary of treated pregnant female rats and that of control pregnant female rats (p < 0.05) (Picture 2).



Picture 2: Relative mass of the ovary in pregnantfemale rats

At the uterinehornlevel, the results obtained show a relative mass of  $0.235 \pm 0.255$  g and  $7.607 \pm 1.298$  g respectively in treated pregnant female rats and control pregnant female rats. Statistical analysis shows that the mean relative mass of the uterinehorn in pregnant female rats treated with 2000 mg/kg body weight of the total aqueous extract of *Calotropis procera* was significantly reduced (p < 0.05) compared to that of pregnant female control rats that received distilled water (Figure 3).

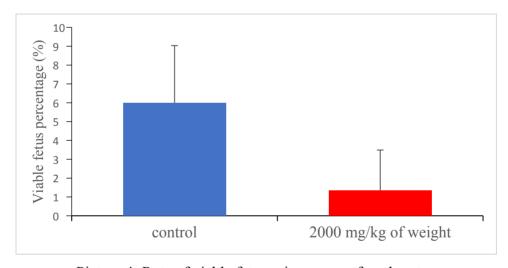
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Picture 3: Relative mass of the uterinehorn of pregnantfemale rats

### Viable fetus rate in treated female rats

The results for the number of viable fetuses in pregnantfemale rats treated with total aqueous extract of Calotropis procera at a dose of 2000 mg/kg body weight gave an average of  $1.33 \pm 2.160$  with a viable fetus rate of 32%. However, in pregnantfemale rats (controls) that received distilled water, the averagewas  $6.00 \pm 3.033$  with a rate of 97.30% viable fetuses. Statistical comparison shows that the rate of viable fetuses observed in treated pregnantfemale rats is significantly lower (p < 0.05) than that observed in control pregnantfemale rats (Picture 4).

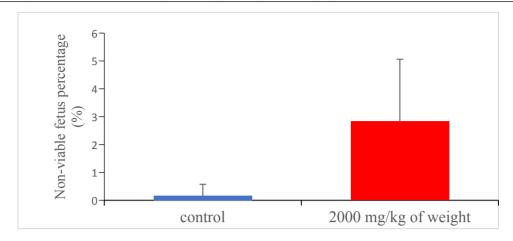


Picture 4: Rate of viable fetuses in pregnantfemale rats

# Rate of non-viable fetuses in treated pregnantfemale rats

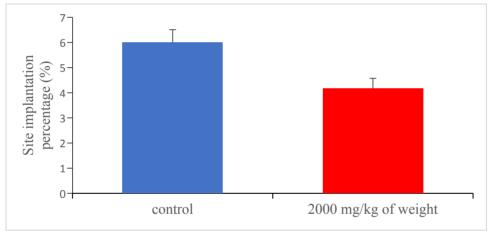
With regard to the rate of non-viable fetuses, the results showed a significantincrease (p < 0.05) in pregnant female rats treated with the total aqueous extract of *Calotropis procera*, which was 68% of non-viable fetuses with an average of 2.833  $\pm$  2.228 non-viable fetuses compared to 0.70% of non-viable fetuses with an average of 0.166  $\pm$  0.4082 non-viable fetuses observed in pregnant female control rats (Picture 5).

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# Sites of implantation in pregnant female rats

The implantation sites in pregnant female rats treated with the total aqueous extract of *Calotropis procera* had a mean of  $4.166 \pm 0.408$  with a rate of 62.5%, compared to the control pregnant female rats, which had a mean of  $6.00 \pm 0.508$  with a rate of 94.8% implantation sites. The statistical study reveals that the implantation site rate in treated pregnant female rats is significantly lower than the implantation site rate in control pregnant female rats (Picture 6).

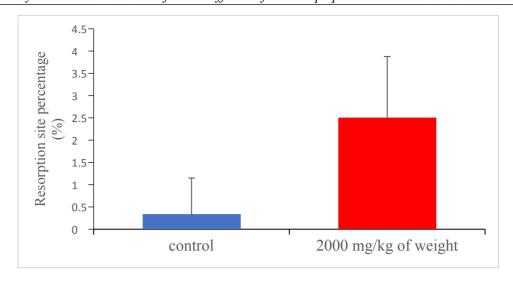


Picture 6: Percentage of implantation sites in pregnantfemale rats

# Resorption sites in pregnantfemale rats

Resorption sites in pregnantfemale rats treated with total aqueous extract of *Calotropis procera* averaged  $2.500 \pm 1.378$  with a resorption site rate of 37.5%, while pregnantfemale control rats averaged  $0.333 \pm 0.8164$  with a resorption site rate of 0.5%. 12% resorption site rate. Statistical studies show that the resorption site rate of pregnantfemale rats treated with the total aqueous extract of *Calotropis procera* is significantly higher (p < 0.05) than that of pregnantfemale control rats (Picture 7).

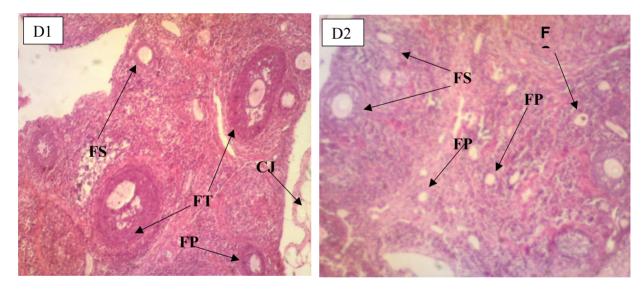
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Picture 7: Resorption rate in pregnantfemale rats

# Histopathology of the ovaries of treated pregnantfemale rats

Analysis of histological sections of the ovaries of control pregnantfemale rats shows severaltertiaryfollicles and corpus luteum compared to the ovaries of treated pregnantfemale rats. In treated pregnantfemale rats, however, the number of primary and secondary folliclesishigherthanthatobserved in control pregnantfemale rats (Picture 8).



Picture 8: Histological section of the ovary of treated pregnantfemale rats

D1: control ovary; D2: treated ovary

CJ: corpus luteum; FP: primary follicle, FS: secondary follicle, FT: follicle

# III. Discussion

Phytochemical screening has shownthat Calotropis procera containsflavonoids, saponosides, catechin tannins, polyphenols, alkaloids, sterols, and polyterpenes. However, Chaidou et *al.*4noted an absence of alkaloids and saponosides in thissame plant. These differences could be explained by the part of the plant used and the location where it was harvested. The leaves used were harvested in Côte d'Ivoire, specifically at Felix HOUPHOUËT BOIGNY University, while the authors used roots harvested in Azawah, a vast area located in northwestern Niger between the Sahara Desert and the Niger River valley.

Alkaloids have estrogeniceffects on the reproductive system of mammals<sup>5</sup>. According to Badgujar and Surana<sup>6</sup>, they have antioxidant, anti-inflammatory, anticonvulsant, and antinociceptive effects<sup>7</sup>.

Flavonoids, in addition to their strongestrogenic actions<sup>8</sup>, regulateestrogen and androgen production<sup>9</sup> in men. Flavonoids have beneficial effects in preventing osteoporosis<sup>10</sup> and on the cardiovascular system by inhibiting the

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oxidation of LDL cholesterol by free radicalsthroughantioxidants<sup>11</sup>. Flavonoidssuch as silymarin, apigenin, quercetin, and naringenin have hepatoprotective and regenerative effects on the liver<sup>12</sup>.

Polyphenols have cardioprotective and anticancer properties<sup>13</sup>. Like polyphenols, polyterpenes have anticancer, apoptotic, and antitumor activities<sup>14</sup>.

Polysterols are wellknown for theireffects similar to those of endogenous steroids. This is the case with  $\beta$ -sitosterol and estrone, highly estrogenic molecules found in palm kernels<sup>15</sup>. They enhance fertility and are used in the treatment of sexual impotence<sup>16</sup>.

Saponins have estrogenic effects<sup>17</sup>. They have the ability to boost testosteronelevels and trigger libido<sup>18</sup>.

Tannins have antibacterial, antifungal, and antiviral properties<sup>19</sup>. Tannins have antioxidant, immunostimulant<sup>20</sup>and antihypertensive<sup>21</sup>properties. Despitethisrichphytochemical composition and promisingpharmacologicaleffects, the use of *Calotropis procera* as an alternative healthcare option requiresscientificstudies.

The body mass gain of treated pregnantfemale rats issignificantlylowerthanthat of control pregnantfemale rats. Zougrou et *al.*<sup>22</sup>obtained a differentresultwith the aqueousextract of Cnestis ferruginea. This difference could be explained by the nutritional value of its extract and also by the number of fetuses carried by each female.

The relative mass of the ovaries of treated pregnantfemale rats did not varysignificantlyfromthat of control pregnantfemale rats. However, the mass of the uterinehorns of treated pregnant rats decreased significantly compared to that of control pregnant rats. This difference could be explained by the number of fetuses contained in the uterine horns<sup>22</sup>. *Calotropis procera* extractappears to reduce the number of fetuses contained in the uterine cavities.

In terms of viable and non-viable fetuses, the number of viable fetusesdecreased significantly in the treated group compared to the control group, and vice versa for non-viable fetuses. These differences could be due to the effect of *Calotropis procera* on embryonic mortality. Faye<sup>23</sup> showed that consumption of high doses of *Calotropis procera* increased the neonatal mortality rate and decreased the number of fetuses compared to control animals.

In terms of implantation sites, the results showed a significant decrease in pregnant female rats treated with the total aqueous extract of *Calotropis procera*. The regular development of all events leading to implantation in mammals is mainly under the direct control of the estrogen-progesterone effect at the cellular level <sup>24</sup>. These results could be due to the effect of the total aqueous extract of *Calotropis procera* leaves, which would prevent implantation, thereby reducing the sites of fetal attachment.

The number of resorption sites increased significantly in pregnant female rats treated with the total aqueous extract of *Calotropis procera* leaves compared to pregnant female control rats. These results are similar to those reported by Zougrou et *al.*<sup>22</sup>, who obtained a significantly higher rate of resorption sites in pregnant female rats treated with aqueous extract of Cnestis ferruginea at a dose of 100 mg/kg body weight compared to pregnant control rats. The significantly higher rate of resorption sites could be explained by the dose used, which may have disrupted the endocrine balance required to maintain pregnancy. The abundance of primary and secondary follicles in treated female rats could be explained by the lowestrogen levels in these rats, which inhibit follicle development.

# **IV.** Conclusion

The phytochemical study of the total aqueous extract of *Calotropis procera* leaves revealed the presence of alkaloids, flavonoids, polyphenols, sterols and polyterpenes, saponosides, and catechin tannins. However, no quinones or gallic tannins were detected.

The total aqueousextract of *Calotropis procera*leaves influences the maturation of primary and secondary follicles and also impacts implantation, i.e., the number of embryoattachment sites (reduces implantation). Histopathology of the organsrevealed no structural changes. This studycouldbefurtherdeveloped by determining the levels of hormones such as estrogen and progesterone in pregnantfemale rats, extending the treatment of pregnantfemale rats untiltheygivebirth, and checking for toxicity in the pups.

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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