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# Preclinical Developmental Toxicity Evaluation of the Aqueous and Ethanolic Extracts of *Ageratum Conyzoides* (Asteraceae) in *Wistar* Rat Models

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## ABSTRACT

**Introduction:** Ageratum conyzoides L. (Family Asteraceae) is an annual herb with a long history of traditional medicinal use in the tropical and sub-tropical region of the world. Its use in pregnancy necessitates a study. Therefore, this study aimed at evaluating A. conyzoides for potential developmental toxicity in female Wistar rats.

**Methodology:** The pregnant rats were treated with 0 mg/kg (only distilled water) or 500 mg/kg of aqueous and ethanolic extracts of the leaves, flowers, stems and combined aerial parts of A. conyzoides. The extracts of the aerial parts of A. conyzoides were administered orally throughout the period of organogenesis of females (5th to the 15th day of gestation). The dams were sacrificed on day 23 or allowed to deliver and wean. Maternal signs of toxicity were monitored throughout the gestational period. The duration of pregnancy, number and weight of neonates, live births and number of dead fetuses were determined immediately after parturition. Each litter was followed up for a period of 28 days post-partum and different parameters related to their growth were recorded.

**Results:** The flower aqueous extract administration to dams induced prolonged labor which lasted 5 to 6 hours. Also, the administration of the aqueous extract of the whole aerial part of A. conyzoides to the dams led to the delivery of no pups. In the treatment (500 mg/kg) groups, no gross abnormalities were observed during offspring examination. The follow-up of the offspring for a duration of 28 days post-partum revealed a significant increase in the weight of the pups from dams delivered by compared to the control group receiving only distilled water.

**Conclusion:** This study has shown that, the administration of the aqueous extract of the combined aerial part of A. conyzoides interrupts pregnancies when administered to dams from the 5th to the 15th day of gestation. In addition, the administration of the flower aqueous extract of A. conyzoides to pregnant rats induces prolonged labor. Also, A. conyzoides extracts do not adversely affect offspring growth following exposure during pregnancy.

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**Keywords:** *Ageratum Conyzoides*, Organogenesis, Developmental Toxicity, Female Rats, Offspring

## Introduction

According to the World Health Organization (WHO), more than 80% of the world's population still rely on herbal medicines as their primary source of health care [1]. In developing countries, there is an upsurge in the use of herbal medicine, especially among rural dwellers for the treatment of human illnesses [2,3]. The reason of such a wide use of medicinal plants has been mainly attributed to their accessibility and affordability [4]. Africans, regardless of their social status or level of education, remain attached to their traditions and often resort to it whenever the need arises. As a result, the belief in the harmlessness of medicinal plants because of their natural origin makes them an alternative to conventional therapies [5].

Plants used in women's health related conditions such as female fertility, menorrhea, birth control, pregnancy, birth, postpartum and lactation, including infant care, have been documented for various ethnic groups [6]. Also, due to the numerous physiological changes that come with pregnancy such as nausea, vomiting, heart burn and constipation, pregnant women tend to resort to self-medication including herbal medicine [7]. Even though plants have been used for centuries by pregnant women, recent scientific studies have shown that they can negatively affect delivery to the

point of likely endangering both maternal and fetal health [8]. For instance, studies in Malawi and Uganda have suggested that traditional medicines may be involved in a significant proportion of maternal deaths [8,9]. Other studies conducted in Douala and Ivory Coast in pregnant women have suggested that they may be involved in maternal and fetal adverse events such as uterine rupture or fetal asphyxia [10,11].

Ageratum conyzoides is an annual herbaceous plant that belongs to the Asteraceae family. Studies have shown that the plant contains a wide range of phytochemicals such as flavonoids, terpenoids, alkaloids, phenols, chromenes, benzofurans and cardiac glycosides [12]. It is widely used in African traditional medicine as purgative, anti-ulcer and wound dressing, measles, pain associated with the navel in children, diarrhea and skin diseases [12,13]. In Cameroon, it is used by pregnant women to facilitate childbirth, swelling of the legs and ankles and as an abortifacient [14,15]. Even though studies have revealed that it contains pyrrolizidine alkaloids known to induce fetal toxicity, little research has been done to investigate its effects on reproduction. Therefore, the present study was undertaken to evaluate the toxicodynamics of A. conyzoides on reproduction in female Wistar rats.

## Materials and Methods

### Plant Material

The plant specimen was identified by a taxonomist in the National Herbarium of Cameroon (NHC) by comparing with reference specimen number 61801/NHC. Fresh mature plants were harvested in Yaoundé in January 2023. The harvested plants were separated into individual aerial parts (leaves, stems and flowers) and combined aerial part of A. conyzoides. The plant material was air-dried separately away from sunlight at room temperature under shade. The plant parts were ground to a fine powder using a milling machine. The fine powder was stored in airtight containers till required.

### Experimental Animals

Ten-week-old male and female Wistar albino rats (male: 370.2–446.9 g; female: 220.4–265.2 g) found to be in good health were selected for use. The animals were fed with a mixture of corn meal (45 %), wheat flour (20 %), fish meal (20 %), soybean meal (10 %), palm kernel (5 %), bone flour for calcium intake (0.98 %), cooking salt (0.5 %) and vitamin complex (0.5 %). The animals were housed in polypropylene rodent cages with free access to water and food. The rats were be randomly selected with respect to body weight for final allotment to the study. The animal house has natural air-conditioned rooms with optimal air changes per hour, relative humidity, temperature and illumination cycles set to 12h light and 12 hours dark.

### Preparation of the Plant Extracts

In this study, aqueous and hydroethanolic extracts of each aerial part (leaves, stem and flowers) as well as the whole aerial part of A. Conyzoides were prepared separately using the maceration technique. In this extraction method, whole or coarsely powdered plant-drug is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved. At the end of extraction, the micelle is separated from marc by filtration and from the menstruum by evaporation in an oven [16].

In this process, 100 g of coarsely powdered plant's leaves, stem and flowers as well as its combined aerial parts, were placed in a stoppered container with 1000 mL of solvent (distilled water, hydro-ethanolic solution 50:50) and allowed to stand at room temperature for a period of 72 hours with frequent agitation until the soluble matter has dissolved. The mixture was then strained, the marc (the damp solid material) pressed, and the combined liquids were clarified by filtration using Whatman paper. At the end of extraction, the micelle is separated from marc by filtration and from the menstruum by evaporation in an oven at 50°C [17]. Each extract of A. conyzoides was stored in an air-tight container for subsequent experimental tests.

### Evaluation of Developmental Toxicity of the Plant Extracts

### Determination of the Estrus Cycle Phase

Vaginal smear analysis test was used to verify the estrus stage of females. A micropipette containing 10 µL of saline solution was gently and superficially inserted into the vaginal cavity of females. The saline solution was injected, aspirated and immediately transferred to a glass slide and examined fresh under light microscopy. The proestrus phase was confirmed by the predominance of cornified cells and absence of leukocytes under microscopic examination [18].

### Mating and Gestation Confirmation

After confirmation of the estrus cycle phase, every female rat determined to be in estrus or pro-estrus phase of their cycles were mated with male rats in the same cage for one day. The next morning, the vaginal smear was redone. The presence of sperms in vaginal cytology or physically observing the presence of a copulatory plug in the vagina of female rats was taken as an indication of successful mating and that day was considered to be Gestational day 0 (GD 0). These pregnant females were separated from the males and placed in individual cages. The day of birth (viz. when parturition is complete) is defined as day 0 post-partum.

### Experimental Design

The OECD 422 Guideline for the testing of chemicals (combined repeated dose toxicity study with the reproduction/developmental toxicity screening test) was applied in this study [19].

### Dosing Range and Grouping Design

Inseminated rats were isolated in their respective cages and GD0 was noted. They were individually weighed and divided into different groups of 8 animals each as follows:

**Group 1:** Control group (received distilled water)
**Group 2:** received the aqueous maceration of leaves of A. conyzoides
**Group 3:** received the hydroethanolic maceration of A. conyzoides leaves
**Group 4:** received the aqueous maceration of A. conyzoides flowers
**Group 5:** received the hydroethanolic maceration of A. conyzoides flowers
**Group 6:** received the aqueous maceration of the stem of A. conyzoides
**Group 7:** received the hydroethanolic maceration of the stem of A. conyzoides
**Group 8:** received the combined aerial part of the plant aqueous maceration of A. conyzoides
**Group 9:** received the combined aerial part of the plant hydroethanolic maceration of A. conyzoides

Each group of animals received the extract of A. conyzoides from GD5 to GD15 (period of organogenesis). The dose administered to all the test groups was 500 mg/kg. This dose was chosen based on previous safety toxicity data such as LD50 above 2 000 mg /kg and repeated dose toxicity studies [20,21].

**Observations**

Once insemination was confirmed, females were checked for signs of parturition from day 19 of pregnancy. General observations were made at least once a day, preferably at the same time(s) each day. The health condition of the animals was equally be recorded. At least twice daily all animals were observed for morbidity and mortality. The pregnant rats were weighed on daily to permit calculation of the extract volume to be administered. To observe maternal toxicity, the following clinical criteria were adopted body weight, piloerection, diarrhea, locomotor activity, vaginal bleeding [22].

Females were allowed to deliver spontaneously and nurse their pups until day 28 of the lactation period. Each litter was examined as soon as possible after delivery to establish the number of pups, stillbirths, live births, runts (pups that are significantly smaller than corresponding control pups), and the presence of gross abnormalities. Live pups were counted and their postural return reflex was recorded immediately. Each litter was weighed within 24 hours of day 0 or 1 post-partum and at least on postnatal day 7, 14, 21 and 28. Each pup's head to tail length was also recorded every 7 days for 28 days. In addition to the observations on parent animals any abnormal behavior of the offspring was recorded. The dams that did not deliver any pups were killed on GD 23 for observation of number of implantations and post-implantation losses.

The weight increase during gestation and weight gain was calculated as follows:

$$\text{Weight gain (g)} = \frac{\text{Weight at the parturition} - \text{initial weight}}{\text{Initial weight}} \text{ x } 100$$

**Statistical Analysis**

The results were expressed in terms of mean ± standard deviation. The comparison between the groups was analyzed using one-way analysis of variance, ANOVA test, followed by Turkey Kramer post hoc multiple comparison test using the GraphPad Instat version 5.0 software. A p-value of less than 0.05 was considered as statistically significant.

**Results**

**Maternal Data**

Copulated female rats were those whose vaginal cytology indicated the presence of spermatozoa or a copulatory plug. Most copulated females survived until the end of the experiment. A total of four dams from both the control and treatment groups died in the study as presented in table 1. Dams treated with the extracts of the plant did not show any sign of maternal toxicity. During the study period, no clinical or behavioral signs indicating systemic, localized toxicity or miscarriage were observed in the groups that received the plant extracts apart from the stem ethanolic and aqueous extracts of A. conyzoides in which two dams in both groups experienced vaginal bleeding from GD13 to 16. In the control, a single dam died during delivery at GD17. This dam experienced vaginal bleeding from GD 14 to GD17. In all, the dams in both control and treatment groups experienced labor between GD 20 and 23.

In both the control and treatment groups (500 mg/kg), dams which reached parturition experienced 1-to-2-hour labour. However, out of the 8 dams which received the aqueous flower extract, 3 dams experienced prolonged and difficult labor which lasted 5 to 6 hours. This led to the delivery of 100% dead pups by these dams. The fetuses remained visibly stuck in the uterus and were eventually resorbed after 3 to 4 days. Some dams by GD 23 delivered no pups as shown in table 1. All the dams which had received the aqueous extract of the combined aerial parts of the plant delivered no pups. Meanwhile, only a part of the dams which received the ethanolic and aqueous stem extracts as well as the ethanolic extract of the combined aerial parts of the plant delivered no pups.

**Table 1: Parameters before Parturition of Copulated Rats of different Groups of Study**

| Groups | Extrants | Meany Wight gain (g) | Mean number of days for parturition | Numbers of damas délivre | Number of dams with no pups | Number of deaths before delivery |
|---|---|---|---|---|---|---|
| Control | | 29,73 ± 4,77 | 20,00 ± 0,00 | 7/8 | 0/8 | 1/8 |
| | Aqueuse | 24,91 ± 8,10 | 21,71 ± 0,76 | 7/8 | 1/8 | 0/8 |
| Lea | | | | | | |
| | Ethanolic | 38,84 ± 9,39 | 20,50 ± 0,84 | 6/8 | 1/8 | 1/8 |
| | Aqueuse | 32,96 ± 12,26 | 21,60 ± 0,89 | 5/8 | 3/8 | 0/8 |
| Stems | | | | | | |
| | Ethanolic | 28,45 ± 6,70 | 20,40 ± 2,07 | 4/8 | 3/8 | 1/8 |
| | Aqueuse | 32,70 ± 8,63 | 20,83 ± 2,64 | 7/8 | 0/8 | 1/8 |
| Flower | | | | | | |
| | Ethanolic | 36,51 ± 3,79 | 20,83 ± 0,98 | 7/8 | 1/8 | 0/8 |
| | Aqueuse | 33,26 ± 12,69 | 00,00 ± 0,00 | 0/8 | 8/8 | 0/8 |
| Plant | | | | | | |
| | Ethanolic | 37,80 ± 21,15 | 22,00 ± 0,00 | 5/8 | 3/8 | 0/8 |

The results are expressed as mean ± SEM

**Mean Weight Gain of Pregnant Animals Subjected to Treatment with Plant Extracts**

The administration of the different extracts to the pregnant animals caused no significant difference in the weight gain between the dams in both control and treatment groups (500 mg/kg) with a p-value > 0.05 was observed as shown in figure 1.

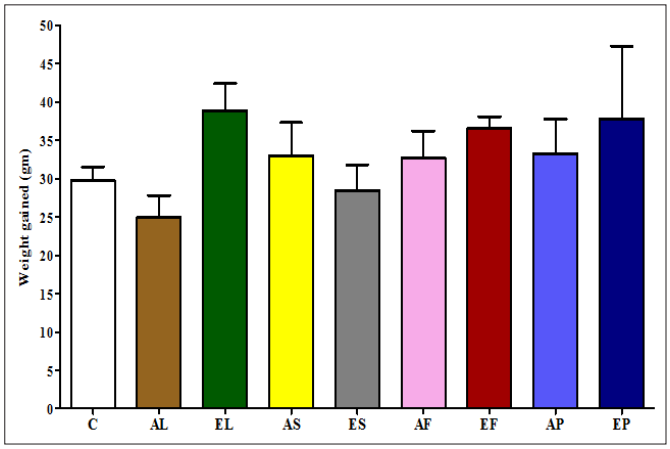


**Figure 1:** Mean Weight Gain of Animals During Gestation

C= control, AL = aqueous leaf, AS= aqueous stem, AF= aqueous flowers, AP = aqueous plant, EL= ethanol leaf, ES= ethanol stem, EF= ethanol flowers and EP= ethanol plant

**Uterine and Litter Observations**

Of the 8 dams in the control group, 1 had a miscarriage and died after excessive bleeding on GD17. The remaining 7 dams in the control group delivered their pups on GD 20 without any stillbirths. In the flower aqueous extract group, 3 out of the 7 dams alive on GD20, experienced prolonged labor and most of the fetuses remained stuck in their uterus. Of the remaining 4 dams, only 1 of them delivered 4 live pups and 3 dead fetuses while the remaining 3 dams delivered only dead pups. The rats which delivered no pup in the groups which received the leaf extracts of the plant were found to be non-pregnant after being sacrificed and uteri were removed. Their uteri showed no sign of preexisting pregnancy. On the other hand, uteri isolated from the rats which delivered no pups following the administration of the aqueous and ethanolic stem extracts of A. conyzoides on GD 23 showed noticeable increase in vascular innervation which is absent in a non-gravid uterus as shown in figure 2. The same was observed in animals which received the aqueous maceration extract of the plant's combined aerial parts.

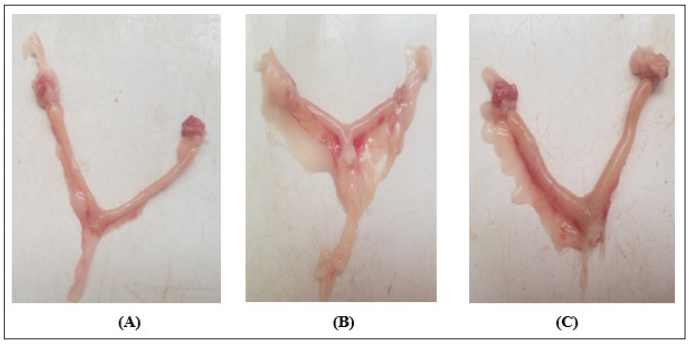


**Figure 2:** Photos of Uteri from Experimental Animals. (Current Study)

A) Uterus from non-gravid virgin rat
B) Uterus from rat which received the combined aerial part of the plant
C) Uterus from rat which received ethanol stem extract

**Effect of Plant Extracts on Litter Size**

The administration of the extracts during the gestational period led to the parturition of the females in the different groups with the exception of the animals which received the aqueous extract of the combined aerial part of the plant. The control group having received only distilled water presented the highest number of births, followed by the hydro-ethanolic extract of the leaves, extracts of the leaves and stems as shown in figure 3. The difference between the groups is non-significant with a p-value > 0.05. However, a significant difference with a p-value < 0.001 between all study groups and the aqueous extract of the combined aerial part of the plant is noted.

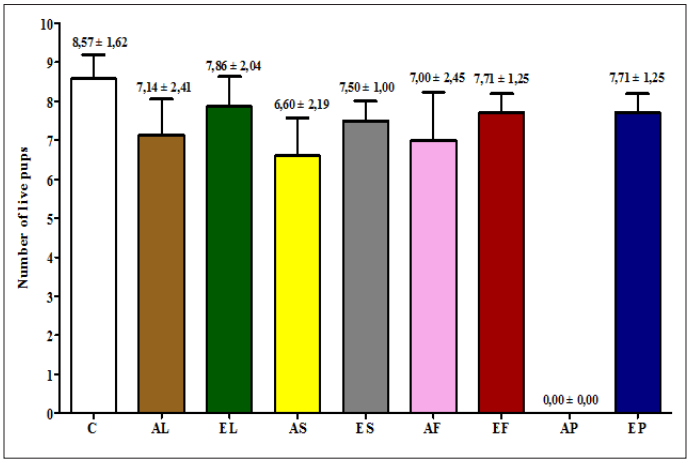


**Figure 3:** Effect of a Conyzoides Extracts on Litter Size

C= control, AL = aqueous leaf, AS= aqueous stem, AF= aqueous flowers, AP = aqueous combined aerial parts of plant, EL= ethanol leaf, ES= ethanol stem, EF= ethanol flowers and EP= ethanol combined aerial parts of Plant

**Effect of the Plant on Offspring**

The pups were followed up till postnatal day 28. They showed no gross abnormalities in both control and treatment (500 mg/kg) groups. Their general development: ear separation, fur appearance and eyes opening weren't affected by administration of the plant's extracts.

**Effect on Litter Survival**

Table 2 shows that there is a non-significant difference in the number of pups which survived for a duration of 28 days post-partum in both control and treatment (500 mg/kg) groups.

**Table 2: Effect of the Plant on Survival of Pups for a Period of 28 Days after Birth**

| Groups | | Mean number of live pups after parturition | | | | |
|---|---|---|---|---|---|---|
| | | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 |
| | | 8 ± 3 | 7 ± 2 | 6 ± 2 | 6 ± 2 | 6 ± 2 |
| | Aqueuse | 7 ± 2 | 6 ± 2 | 7 ± 2 | 6 ± 2 | 6 ± 2 |
| Lea | Ethanolic | 7 ± 2 | 6 ± 3 | 5 ± 2 | 6 ± 2 | 6 ± 2 |
| | Aqueuse | 6 ± 2 | 6 ± 2 | 6 ± 2 | 6 ± 2 | 6 ± 2 |
| Stem | Ethanolic | 7 ± 1 | 7 ± 1 | 7 ± 1 | 7 ± 1 | 7 ± 1 |
| | Aqueuse | 7 ± 2 | 6 ± 1 | 6 ± 1 | 6 ± 1 | 6 ± 1 |
| Flower | Ethanolic | 7 ± 1 | 7 ± 1 | 7 ± 1 | 7 ± 1 | 7 ± 1 |
| CAP | Ethanol | 7 ± 1 | 6 ± 1 | 5 ± 1 | 5 ± 1 | 6 ± 1 |

CAP: Combined Aerial Parts. The results are expressed as mean ± SEM

**Effect on Litter Growth**

**Body Weight Evolution**

The table 3 shows the weight evolution of the pups after parturition for a period of 28 days. When comparing the weight of pups from the control and treatment (500 mg/kg) groups on each postnatal day (day 1, 7, 14 and 28), no conspicuous significant difference between the groups was observed. However, on day 14, 21 and 28, an exception was observed with the pups from dams which had received the stem aqueous extract. It was noted that the body weight of these pups on those days were significantly high when compared to the control and other treatment (500 mg/kg) groups in the study.

**Table 3: Evolution of the Weight of Pups in the different Study Groups for a Period of 28 Days**

| Groups | | Wight (g) | | | | |
|---|---|---|---|---|---|---|
| | | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 |
| | | 4,56 ± 0,35$^{d,i,l,q,u,x}$ | 8,08 ± 1,38$^{j,r,u,x}$ | 14,83 ± 4,25$^{e,l,u,w}$ | 21,38 ± 5,89 | 34,84 ± 9,95 $^{l}$ |
| | Aqueuse | 4,89 ± 0,55$^{x}$ | 9,49 ± 2,07$^{s,t}$ | 15,87 ± 4,18$^{l,t}$ | 22,55 ± 6,11 | 32,68 ± 7,56 |
| Lea | Ethanol | 5,15 ± 0,55$^{x}$ | 10,06 ± 2,15$^{s,x}$ | 17,98 ± 3,52$^{m}$ | 27,99 ± 4,12 | 41,10 ± 6,11 |
| | Aqueuse | 5,12 ± 0,36$^{v}$ | 11,50 ± 1,77$^{c}$ | 21,73 ± 3,27 | 31,72 ± 6,15 | 47,34 ± 10,72 |
| Stem | Ethanol | 4,89 ± 0,39$^{x}$ | 9,64 ± 1,39$^{w}$ | 17,15 ± 2,68l | 23,94 ± 4,13 | 37,25 ± 5,91 |
| | Aqueuse | 5,00 ± 0,41$^{x}$ | 11,60 ± 1,82 | 16,90 ± 4,32$^{k}$ | 23,21 ± 7,81 | 35,36 ± 11,84 |
| Flower | Ethanol | 5,17 ± 0,47$^{w}$ | 11,62 ± 2,01 | 18,89 ± 2,31 | 26,04 ± 3,81 | 35,99 ± 7,39 |
| Plant | Ethanol | 5,60 ± 0,73 | 12,21 ± 1,61 | 18,19 ± 2,99 | 26,25 ± 4,56 | 37,66 ± 4,99 |

Values are expressed in terms of mean ± standard deviation, (n = 3). The comparison between groups is made using the variance test (ANOVA) followed by the Turkey Kramer post hoc test. the difference is significant with a < 0.05; b < 0.01; c < 0.001 for control; d<0.05; e < 0.01; f < 0.001 for aqueous leaves; g < 0.05; h < 0.01; i < 0.001 for ethanolic leaves; j < 0.05; k < 0.01; l < 0.001 for aqueous stem extract ; m < 0.05; n < 0.01; o < 0.001 for ethanolic stem extract; p < 0.05; q < 0.01; r < 0.001 for aqueous flower extract; s < 0.05; t < 0.01; u < 0.001 for ethanol flower extract; v < 0.05; w < 0.01; x < 0.001 for the combined aerial parts of the plant ethanol extract.

**Head-To-Tail Length Evolution**

The table 4 presents the evolution of the head-tail distance of pups from the different study groups from day 1 to 28 after birth. It was observed that the growth of the pups in the control and treatment (500 mg/kg) groups are not remarkably different when compared on day 1, 7, 14 and 28.

**Table 4: Evolution of the Head to Tail Length of Pups for a Period of 28 Days after Parturition**

| Groups | | Length (cm) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 | 28 |
| Control | | 4,15 ± 0,25$^{i,u}$ | 5,13 ± 0,88$^{f,i,l,o,r,u}$ | 6,52 ± 0,60$^{f,l,o,r,u}$ | 8,31 ± 0,75$^{j,u}$ | 9,84 ± 0,74$^{h,l,o,r,u}$ |
| | Aqueuse | 4,19 ± 0,23$^{u}$ | 5,73 ± 0,48$^{q,s}$ | 6,55 ± 0,51$^{i,l,o,r}$ | 8,37 ± 1,19$^{j,u}$ | 9,80 ± 1,22$^{i,l,o,r,u}$ |
| Lea | Ethanolic | 4,36 ± 0,32$^{x,s}$ | 5,79 ± 0,43$^{p}$ | 7,47 ± 0,80$^{u,t}$ | 8,84 ± 0,67$^{t}$ | 10,74 ± 0,66 |
| | Aqueuse | 4,30 ± 0,27$^{v,r}$ | 5,85 ± 0,41$^{c}$ | 7,78 ± 0,34 | 9,00 ± 0,53$^{s}$ | 11,28 ± 1,06 |
| Stem | Ethanolic | 4,19 ± 0,19$^{u}$ | 5,68 ± 0,40$^{q,s}$ | 7,52 ± 0,55$^{u}$ | 8,80 ± 0,58 | 10,92 ± 0,48 |
| | Aqueuse | 4,20 ± 0,25$^{u}$ | 6,26 ± 0,41 | 7,44 ± 1,01$^{t}$ | 8,96 ± 1,07$^{t}$ | 10,91 ± 1,17 |
| Flower | Ethanolic | 4,54 ± 0,23$^{w}$ | 6,09 ± 0,47 | 8,09 ± 0,53 | 9,61 ± 0,72 | 11,31 ± 0,58 |

Values are expressed in terms of mean ± standard deviation, (n = 3). The comparison between the groups is carried out using the test of variance (ANOVA) followed by the Turkey Kramer post hoc test. The difference is significant with a < 0.05; b < 0.01; c < 0.001 for control ; d<0.05 ; e < 0.01; f < 0.001 for aqueous leaf extract ; g < 0.05; h < 0.01; i < 0.001 for ethanolic leaf extract ; j < 0.05; k < 0.01; l < 0.001 for aqueous stems; m < 0.05; n < 0.01; o < 0.001 for ethanolic stems; p < 0.05; q < 0.01; r < 0.001 for aqueous flower extract; s < 0.05; t < 0.01; u < 0.001 for flower ethanol; v < 0.05; w < 0.01; x < 0.001 for the ethanol extract of combined aerial parts of the plant.

## Discussion

Many medicinal plants are used by pregnant women for their therapeutic effects. However, these plants are consumed mostly based on personal experience or traditional knowledge and in most cases, it is unclear how safe their use is during pregnancy [23]. A. conyzoides from the Asteraceae family is among the plants which are most commonly used by pregnant women in several ethnicities. However, some phytochemicals known to possess adverse effects on reproductive health of women have been found in this plant especially pyrrolizidine alkaloids [24]. Studies on some other plants also belonging to the Asteraceae family such as Artemisia lanata, Artemisia annua, Achillea millefolium and Aspilia Africana have demonstrated that these plants affect the reproductive health of female rats [23,25]. It is therefore necessary to consider that A. conyzoides belonging to this family of plants, widely used traditionally by pregnant women for the treatment of several ailments, could also pose a threat to female reproductive health.

In order to evaluate prenatal toxicity induced by plants or their active compounds, many factors were considered, such as the period of pregnancy, the time of exposure, and the doses administered, among others. In this study, the plant was administered to dams from the start of organogenesis, that is, from the 5th to the 15th gestational day, characterized by the growth and differentiation of tissues into organs [26]. During the study period, the extracts of the A. conyzoides did not induce maternal toxicity. The plant extract administration did not equally affect the gestational length of the dams. This concurs with the findings of Maciela in a study conducted on the flower extracts of Achyrocline satureioides (Asteraceae) [27].

Generally, in rats, the implantation window corresponds to a period of approximately 24 h between day 4 and day 5 of pregnancy. During this phase, the endometrium undergoes morphological modifications induced by estrogen and progesterone that facilitate apposition, attachment and embryo invasion [28]. In this study, a part of the dams that received ethanolic and aqueous stem extracts of A. conyzoides, delivered no pup meanwhile none of the dams that received the aqueous extract of the combined aerial part of the plant delivered any pups. Upon sacrificing some rats on GD23, no fetuses were found although there was marked increase in uterine vascularization. This change in uterine vasculature might be related to a preexisting pregnancy which might have been interrupted at an early stage immediately after implantation as observed in a study conducted by Camilleri [29]. The stem of mature A. conyzoides makes up the greatest part of its aerial part which might explain why the administration of extracts of the combined aerial parts to dams resulted in effects which were in a closely similar manner to the stem extracts of the plant in this study.

Labor dystocia was observed in groups that received the aqueous flower extract of the plant. In this group, the dams experienced prolonged labor and most of the fetuses remained visibly stuck in their uterus. This led to the delivery of dead pups by the dams. Normally, prostaglandins E2 and F2a tend to induce uterine contractions for expulsion of the fetus. The prolonged labor observed could be due to low prostaglandins levels probably caused by administration of the extract [30]. This corroborates with the findings of Henri in a study conducted to investigate the effects of the several plants including those from the Asteraceae family during pregnancy in women [5]. Also, A. conyzoides methanol extract showed the capacity to inhibit uterine contractions induced by 5-hydroxy tryptamine in a study conducted by Lans [31].

The pups from both control and treatment groups were followed up until the 28th postnatal day. They showed no gross abnormalities in both control and treatment groups. It was noted that the administration of the plant extracts to the dams did not result in a remarkable difference in the weight of the pups when compared to the control. However, from the 14th to the 28th postnatal day, the weight of pups from dams which had received the stem aqueous extract consistently was remarkably high when compared to the other treatment (500 mg/kg) and control groups. This is in agreement with the findings of Maciel [27]. The mechanisms underlying the association between pup high weight and A. conyzoides exposure during pregnancy is still unclear, but may be related to elevation in TSH (thyroid stimulating hormone) levels exhibited in treated-females, as maternal thyroid hormones supply the fetus with this hormone throughout pregnancy, and play a critical role in fetal growth and neurodevelopment according to the work conducted by Wang [32]. Also, the pups delivered by dams which had received the ethanol extract of the combined aerial parts of the plant had the highest weight in comparison to all the other treatment groups on the first day of birth. This is contrary to what was observed in the study conducted by Diallo on the effect of the ethanol leaf extract of A. conyzoides administered to dams from the 17th to the 20th gestational day which reported

that the ethanol leaf extract rather provokes a decrease in fetal weight [13]. This might be linked to the fact that the extracts were administered at a different gestational stage.

## Conclusion

In all, this study has shown that the administration of the aerial part of A. conyzoides aqueous extract could interrupt pregnancies when administered to dams from GD5 to GD15. Meanwhile, the administration of the flower aqueous extract of A. conyzoides to pregnant rats induces prolonged labor. The present study also demonstrated that A. conyzoides aqueous and ethanolic extracts of the combined aerial parts as well as the individual aerial parts of the plant neither induce maternal toxicity nor provoked visible abnormalities to the offspring. The different A. conyzoides extracts administration doesn't equally negatively affect offspring growth following exposure during pregnancy.

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## Ethical Considerations

The study was conducted after having approval from the institutional review board of the Faculty of Medicine and Biomedical Sciences. An authorization was obtained from the head of Laboratory for preclinical animal studies and pharmaco-toxicology research.

## Authors Contribution

NBMO, ETF, TYO and FCN conceived, designed the study and drafted the manuscript. MGA, BHN, NNBL and NBMO coordinated laboratory analysis and data assembly. NBMO and NNBL did the data mining. All authors read and reviewed the final draft of manuscript for publication.

## Consent

It is not applicable.

## Conflicts of Interest

The authors declare no conflict of interest.

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