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Maternal-fetal repercussions of *Angylocalyx oligophyllus* leaves aqueous extract in pregnant rat

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Angylocalyx oligophyllus **(Fabaceae) is a shrub used traditionally to treat diabetes mellitus and intestinal parasites. Although it is also used by pregnant women, no scientific study has yet revealed its effects on pregnancy. This work aimed to assess the toxic effects of *A. oligophyllus* leaves aqueous extract on pregnancy, reproduction and fetal development in pregnant rats. The acute toxicity of the *A. oligophyllus* leaves aqueous extract was firstly performed in female non-pregnant rats. Then, pregnant rats were divided into a control and three test groups receiving, respectively distilled water and *A. oligophyllus* leaves aqueous extract doses (50, 100 and 200 mg/kg) by gavage for 20 days (from pregnancy screening day). The daily body masses of pregnant rats and the 21st-day relative organs masses were measured for assessing the pregnancy progress. The numbers of corpora lutea, implantation sites, live and dead fetuses and calculated pre-and-post implantation loss for appreciating the reproduction, and the fetuses' masses for fetal development assessment, were recorded on the 21st day. Acute administration of the *A. oligophyllus* leaves aqueous extract (2000 mg/kg) did not cause any death or adverse effect in non-pregnant female rats. The LD_{50} was estimated higher than 2000 mg/kg. Pregnancy and reproductive parameters did not vary significantly between plant extract-treated rats and control. However, although fetal development parameters did not change significantly between the groups, the percentages of small (SGA) and large (LGA) pups for the gestational age were higher in rats treated with the dose extract of 200 mg/kg, compared to control (20 and 11%, respectively). Current data showed that the *A. oligophyllus* leaves aqueous extract does not impair motherhood and reproduction. Nevertheless, limitation of the dose is recommended during treatment in pregnant women to avoid adverse effect on fetal development.**

Key words: *Angylocalyx oligophyllus*, toxic effects, pregnancy, embryo-fetal development.

INTRODUCTION

Medicinal plants are an important source of natural active compounds with a range of impressive pharmacological activities. Thus, in low- and middle-income countries, 80% of the population resorts to traditional medicine for

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their health problems because of their accessibility, availability and sometimes affordable costs (Baker et al., 1999; Mills and Bone, 2000; Telefo et al., 2002; Ganguly et al., 2007; Cherdshewasart et al., 2007). Several of these plant species are recognized in traditional medicine to have beneficial properties on fertility and pregnancy. Several studies have already proven the fertile potential and the preventive character against various diseases in pregnancy, of some medicinal plants as *Tribulus terrestris* (Dakshayini and Mahaboob, 2018), *Caralluma dalzelii* (Ugwah-Oguejiofor et al., 2020), and *Phyllanthus niruri* (Paula et al., 2020); Others, however, have been reported to have abortifacient effects such as *Croton unucurna* (Moraes-Souza et al., 2017) and *Trigonella foenum-graecum L.* (Oufquir et al., 2020). Nevertheless, many other plants have not yet been the subject of proper scientific studies on both their therapeutic and toxic potential.

Species of the genus *Angylocalyx* are used empirically because of possible hypoglycemic or antidiabetic, antiparasitic (intestinal parasites and filariasis), antibacterial (gonorrhea), cell restorer, and lactation improver effects. The species *Angylocalyx oligophyllus* Baker (Fabaceae) used even by pregnant women in Cameroon (for smooth running of pregnancy), is a shrub of Africa tropical forests widely distributed in Angola, Benin, Cabinda, Cameroon, Central African Republic, Congo, Equatorial Guinea, Gabon, Ghana, Guinea, Ivory Coast, Liberia, Nigeria and Zaïre. It is known as elongo or etongo (Baka pygmees, Cameroon), iboa, biem, sumba, nvuti, etc. (in Congo) (Letouzey, 1966; Burkill, 1995; Neuwinger, 2000). *A. oligophyllus* was the subject of a recent study describing the presence of phytochemicals classes such as flavonoids, alkaloids, inositol, saponins and sterols in leaves Methyl Chloride/Methanolic extract and its fractions. Many compounds were even identified including formononetin (an isoflavone), stigmasterol-3-O-β-D-glucopyranoside (alkaloid) and pinitol (inositol) (Wakeu et al., 2018).

Data on biological (therapeutic and/or toxic) effects of phytochemicals' classes and metabolites are documented and allowed to explain the action mechanisms of plants extracts. The foeto-maternal beneficial plants effects have often been linked to the presence of flavonoid, saponin, sterol and polyphenol phytochemicals with antioxidant, anabolic, fertile, hormone-regulating properties, etc. (Simionatto et al., 2007). It is the case for example of formononetin reported to have estrogenic, anti-hypertensive, relaxant activities (Sun et al., 2011), and pinitol, saponins and alkaloids which would have anti-diabetic and anti-inflammatory properties (Pandi et al., 2022). On the other hand, the foeto-maternal harmful effects of plants have been often attributed to the presence of alkaloids and terpenes whose abortifacient properties are well known (Hwa et al., 2019). The pharmacological data on *A. oligophyllus* plant concerns only the *in vitro* antidiabetic (reduces α-glucosidase and α-fucosidase) and antioxidant (scavenges free radicals) properties of its extracts, fractions and some identified metabolites, from leaves (Wakeu et al., 2018) and stem bark (Wakeu et al., 2022). However, there is no yet pharmacological study assessing it adverse biological effects on the female reproductive system affecting fertility and reproductive capacity (endocrine system, pregnancy, parturition, lactation).

In order to verify the hypothesis whether *A. oligophyllus* could cause maternal or embryo-fetal deleterious effects (congenital malformations, growth retardation, death and post-natal function deficits in fetus), the present study aimed to assess the toxic effects of *A. oligophyllus* leaves aqueous extract on maternity, reproductive parameters and fetal development in pregnant rats.

MATERIALS AND METHODS

The study was conducted in respect of all guidelines for care and use of Laboratory animals as described in the European Community Guidelines (EEC directive 2010/63/EU of the September 22, 2010) and after obtaining approval for animal experimentation no. 2454 CEI-UDo/08/2020/M.

Chemicals

Accu-chek Active blood glucose test strips and glucometer from Roche Diagnostics (Mannheim, Germany) and all other reagents and chemicals for biochemical analyses (Extra pure analytical grade) from common commercial suppliers were used in the study.

Animal material

Females (non-pregnant and pregnant) and males albino Wistar rats aged 10 to 12 weeks and weighing an average of 160 ± 20 g were used in this study. They were brought up in the animal facility of Laboratory of Biology and Physiology of the Faculty of Science and the University of Douala, housed in cages lined with shavings (2 rats per cage). They were subjected to a natural lighting cycle (12 h day/12 h night) and room temperature. They were fed with dried pellets of food consisting of ingredients from local market and mixed in the following proportions for 100 g of mixed food (50% corn meal, 4% wheat flour, 20% fish meal, 10% corn peanut flour, 3% bone meal, 4% wheat flour, 7% palm kernel oil, 2% salt and a vitamin complex of 0.02%), and tap water.

Plant material

The leaves of *A. oligophyllus* were harvested in the Center Region of Cameroon (Song-Bong at 56 Km from Eseka,) in February 2021. The plant of *A. oligophyllus* was authenticated in the national herbarium of Yaoundé, by comparison with the sample No. 55817/HNC. The leaves were dried and then ground into powder.

Extraction procedure

As recommended by the traditional healer, the *A . oligophyllus*

leaves aqueous extract was prepared by boiling dry leaf powder (366.6 g) in distilled water (3L) for 40 min. Then, the hot mixture which was occasionally shacked for an hour at room temperature (24±2°C) until cooling, was filtered using firstly two fine mesh sieves and then Whatman paper No. 3. The residue obtained was again macerated and filtered following the same procedure. The two filtrates thus obtained were mixed and concentrated by evaporation using an oven at 40°C. After evaporation, an approximately 11.813 g of dry *A. oligophyllus* aqueous extract (3.22%) was obtained and stored at -20°C until use.

Acute oral toxicity test

The acute toxicity test was carried out according to the recommendations of guideline no. 423 of the OECD (2001). Briefly, six female rats used were divided into two groups of three rats each as:

Group 1 or Control: consisting with rats receiving the distilled water (10 mL);
Goups 2 or AoAE 2000: rats treated with *A. oligophyllus* leaves aqueous extract at a dose of 2000 mg/kg.

Animals were administered with single dose of distilled water or extract, respectively, and observed continuously for 4 h, then every 24 h for the 7 days after, and finally once at the end of the 7 following last days, periods during which toxicity signs, deaths and body masses were recorded. After 14 days, the surviving animals were anesthetized by intramuscular injection of ketamine (10 mL/kg), then dissected. The main detoxification organs (liver, lungs and kidneys) and other organs were collected and weighed for relative organ weight determination.

Mating procedure and experimental groups

Vaginal smear of adult female rats was collected every morning to identify the different phases of estrous cycle. All female rats at the end of proestrus were subjected overnight to adult male rats to be fertilized (mating period). The morning when spermatozoa were found in the vaginal smear was designated as day 0 of pregnancy. The mating procedure could extend over a maximum of 15 days, that is, approximately three estrous cycles after which non-fertilized female rats in this period were considered infertile and removed from the study (Damasceno et al., 2011). After mating period, the fertilized female rats considered as pregnant rats were randomly distributed into four experimental groups (n=6 pregnant rats / group) as:

Group 1 or Control: consisting with pregnant rats receiving the distilled water;
Groups 2 - 4 or AoAE 50, AoAE 100 and AoAE 200: consisting with pregnant rats treated with *A. oligophyllus* at doses 50, 100 and 200 mg/kg respectively.

The treatments (distilled water and AoAE or *A. oligophyllus* aqueous extract doses) were administered once a day in the morning by gavage for 20 days. The dosage selection of AoAE was based on the dose empirically administered by the traditional healer, and the therapeutic dose determined during the acute toxicity test (OCDE, 2001).

Course of pregnancy

During pregnancy, maternal weight was measured daily, at approximately 9 a.m. On the morning of day 20 of pregnancy, pregnant rats were individually placed in metabolic cages for 12 h, for urine collection according to the method of Barros et al. (2006), and urine creatinine measure. At day 21 of pregnancy, the rats were anesthetized by diazepam/ketamine (70/30; in Intramuscular), then decapitated and blood samples were collected for blood form counts (NFS) and serum biochemical parameters. The animals were then submitted to laparotomy and uterine horns and other organs (Liver, kidney, spleen, heart, lung…) were collected and weighed.

Organs macroscopic (relative weight) and structural (kidneys) analyses

The relative weights of organs (liver, kidney, heart, lung, spleen …) were calculated by ratio of each organ weight on the 21st day body weight of pregnancy minus the gravid uterus weight. On the other hand, the kidneys were fixed in 10% formaldehyde, then dehydrated, impregnated and included in paraffin blocks which were then cut into 4 µm slices mounted on histological slides. The organ slices thus mounted on slides were then deparaffinized, rehydrated and stained with hematoxylin-eosin, and finally observed under a light microscope for structural analyze.

Hematological analysis

The red blood cells number (RBC), white blood cells (WCB), hemoglobin rate (HGB), hematocrit (HCT), platelets (PLT), mean globular volume (MGV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin level (MCHL) were measured using automated hematology analyzer (URIT- 3000 PLUS).

Maternal biochemical parameters analysis

Collected maternal whole blood was centrifuged at 3000 ×g and the serum obtained was stored at - 20°C for total protein, creatinine and urea levels measurement, as well as Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities, using colorimetric or kinetic commercial kits.

Reproductive outcomes and fetal development

Removed gravid uterus was dissected for determining live and dead fetuses' number, reabsorption rate (embryonic death), numbers of implantation sites and luteal bodies. The number of undetectable implantation sites was determined as described by Costa-Silva et al. (2007). The rate of pre-implantation loss was calculated as ((number of corpora lutea – Number of implantations) × 100 / Number of implantations) (Santos et al., 2015). Collected fetuses from the uterine horns were weighed for body weight classification according to the mean ± 1.7 × standard deviation (SD) of body weight obtained in the control group (Soares et al., 2018).

Statistical evaluation

For comparison of the mean values among the experimental groups, One-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test was used. Subsequently a "Two-way ANOVA" with Bonferroni's post-test was used only to establish the weight gain difference between groups. Differences were

Table 1. Effects of single administration of *A. oligophyllus* leaves aqueous extract on some behavioral and physiological parameters in non-pregnant rats.

Group	Parameters	Times											
		1h	2h	3h	4h	J_1	J_2	J_3	J_4	J_5	J_6	J_7	J_{14}
Control Dist. Water (10 mL/kg)	Grooming	N	N	N	N	N	N	N	N	N	N	N	N
	Pelage	N	N	N	N	N	N	N	N	N	N	N	N
	Mobility	N	N	N	N	N	N	N	N	N	N	N	N
	Reaction to noise	N	N	N	N	N	N	N	N	N	N	N	N
	Stool appearance	G	G	G	G	G	G	G	G	G	G	G	G
	Trembling	-	-	-	-	-	-	-	-	-	-	-	-
	Number of Deaths	0	0	0	0	0	0	0	0	0	0	0	0
A. oligophyllus Aqu. Extract (2000 mg/kg)	Grooming	N	N	N	N	N	N	N	N	N	N	N	N
	Pelage	N	N	N	N	N	N	N	N	N	N	N	N
	Mobility	N	N	N	N	N	N	N	N	N	N	N	N
	Reaction to noise	N	N	N	N	N	N	N	N	N	N	N	N
	Stool appearance	G	G	G	G	G	G	G	G	G	G	G	G
	Trembling	-	-	-	-	-	-	-	-	-	-	-	-
	Number of Deaths	0	0	0	0	0	0	0	0	0	0	0	0

N= Normal; - = No; G=granular
Source: Experience Results | Generated with Office Word

considered statistically significant for $p<0.05$.

RESULTS

Acute toxicity of *A. oligophyllus* leaves aqueous extract in non-pregnant female rats

The single oral dose of 2000 mg/kg of *A. oligophyllus* leaves aqueous extract caused no significant behavioral changes in non-pregnant rats. No death was observed during the first 4 h following the administration of the extract, or during the 14 days afterwards (Table 1). Moreover, the body and relative organ weights of treated rats did not vary compared to the control during the 14 days following the administration (Figure 1 and Table 2).

Effects of *A. oligophyllus leaves aqueous extract* administration on weight change and relative organ weight of pregnant rats

Figure 2 shows that the body masses of the pregnant rats significantly increased from days 15 to 20 of pregnancy in all groups compared to Day 0 ($p<0.05$ - $p<0.0001$) (Figure 1). The body mass increase did not change between the groups until the end of pregnancy.

Furthermore, only the relative mass of the spleen of pregnant rats treated with the plant extract dose of 50 mg/kg increased significantly ($p<0.05$) compared to the control pregnant rats (Table 3).

Effects of the *A. oligophyllus* leaves aqueous extract treatment on blood form count of pregnant rats

The administration of *A. oligophyllus* leaves aqueous extract (50 – 200 mg/kg) to pregnant rats did not alter hematology parameters compared to control pregnant rats (Table 4).

Reproductive outcome of rats treated with different doses of *A. oligophyllus* leaves aqueous extract during pregnancy

The pregnant rats receiving the *A. oligophyllus* leaves aqueous extract showed the gestation percentage of 100% at all doses (50, 100 and 200 mg/kg), against 90% for the control pregnant rats. The number of corpora lutea, implantation, resorptions and pre- and post-implantation loss, the gravid uterus weight, the birth index and the live fetuses' number did not significantly vary between the *A. oligophyllus* leaves aqueous extract-treated and control groups. Furthermore, the proper mother weight gain at day 20 (maternal weight minus the gravid uterus weight) did not differ between groups; no dead fetus was also observed in groups (Table 5).

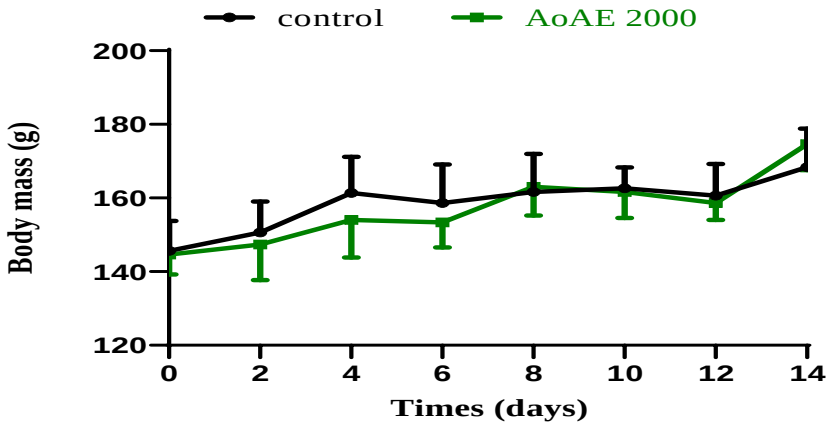

Figure 1. Effects of single dose of *A. oligophyllus* leaves aqueous extract on body mass in non-pregnant rats. Values are expressed as mean ± ESM; n = 3; AoAE 2000 = *Angylocalyx oligophyllus* aqueous extract at 2000 mg/kg; control received distilled water (10mL/kg). No significant difference compared to control.
Source: Experience Results | Graphpad Prism 8.4

Table 2. effects of *A. oligophyllus* leaves aqueous extract on relative organs masses.

Parameter		Groups	
		Control (DW: 10 mL/kg) (n = 3)	AoAE 2000 mg/kg (n = 3)
Heart		0.30±0.00	0.30±0.02
Lungs		0.71±0.10	0.53±0.05
Liver		3.2±0.10	3.2±0.10
Spleen		0.27±0.02	0.29±0.04
Uterus		0.20±0.02	0.26±0.02
Ovary	Right	0.12±0.01	0.031±0.01
	Left	0.030±0.00	0.027±0.01
Adrenal	Right	0.018±0.00	0.016±0.00
	Left	0.018±0.00	0.019±0.00
Kidney	Right	0.28±0.01	0.26±0.02
	Left	0.26±0.01	0.26±0.02

Values are expressed as mean ± ESM; AoAE= *Angylocalyx oligophyllus* aqueous extract; DW = distilled water.
Source: Experience Results | Generated with Office Word

Development of fetuses from female rats treated with *A. oligophyllus* aqueous extract during pregnancy

The mean mass of fetuses from *A. oligophyllus* leaves aqueous extract-treated rats was not significantly different compared to fetuses from control mothers. Moreover, these masses were majorly adequate to gestational age in all groups (Table 6). Compared to the control pregnant females, the percentage of pups with a small mass for gestational age (SGA) decreased by half in those treated with plant extract doses of 50 and 100 mg/kg but doubled at the dose of 200 mg/kg. Furthermore, the three plant extract doses showed high percentages of pups with a large mass for gestational age (LGA), with a maximum percentage observed at the dose of 200 mg/kg, compared to controls.

Biochemical profile and kidney histomorphology of pregnant rats treated with *A. oligophyllus* aqueous extract

Table 7 shows that the administration of the of *A. oligophyllus* leaves aqueous extract (at all doses) during pregnancy did not alter significantly the serum levels of total proteins, transaminases (ALT and AST), urea and

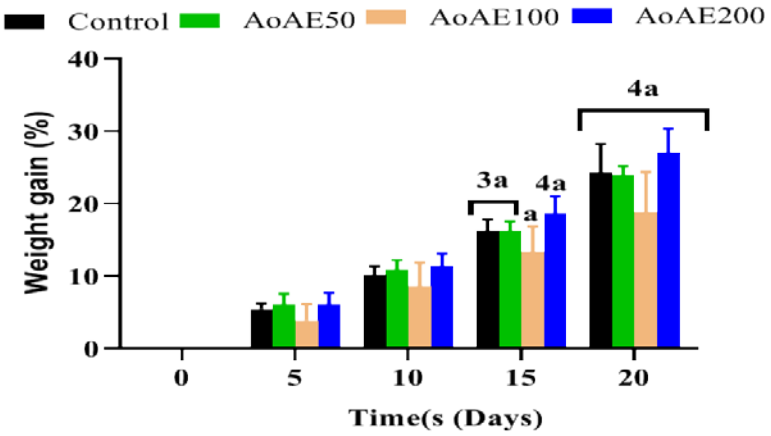

Figure 2. Body or maternal weight gain in pregnant rats treated with *A. oligophyllus* leaves aqueous extract. Values are expressed as mean ± ESM; n = 6; [a]p<0.05, [3a]p<0.001, [4a]p<0.0001 = significant difference from Day 0; AoAE 50, AoAE 100 and AoAE 200 = *Angylocalyx oligophyllus* aqueous extract at doses indicated.
Source: Experience Results | Graphpad Prism 8.4

Table 3. Relative weight of organ with *A. oligophyllus* leaves aqueous extract.

Organs	Groups			
	Control	AoAE50	AoAE100	AoAE200
Heart	0.24 ± 0.01	0.24 ± 0.01	0.22 ± 0.01	0.24 ± 0.01
Liver	2.60 ± 0.04	2.60 ± 0.04	2.70 ± 0.22	2.50 ± 0.07
Kidney	0.2 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.22 ± 0.02
Ovaries	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Lungs	0.52 ± 0.04	0.56 ± 0.04	0.48 ± 0.04	0.48 ± 0.03
Spleen	0.16 ± 0.01	0.25 ± 0.03 *	0.18 ±0.01	0.19 ± 0.02
Placenta	3.60 ± 0.50	3.70 ± 0.40	4.00 ± 0.30	3.90 ± 0.20
Abdominal fat	1.20 ±0.20	0.90 ± 0.20	1.00 ± 0.30	1.30 ± 0.20
Peri-gonadal fat	1.60 ± 0.10	1.60 ± 0.20	1.60 ± 0.10	1.90 ± 0.10
Adrenal glands	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00

Values are expressed as mean ± ESM; n = 6; *p <0.05 = significant difference from control; AoAE 50, AoAE 100 and AoAE 200 = *Angylocalyx oligophyllus* aqueous extract at doses indicated.
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creatinine compared to the control. On the other hand, the urine creatinine level significantly (p<0.05) decreased in pregnant rats treated with the dose extract of 50 mg/kg compared to control. Moreover, the *A. oligophyllus* leaves aqueous extract did not alter the renal tissue compared to control (Figure 3).

DISCUSSION

Traditional medicine remains an important source of effective drugs for the management of many pathologies in low- and middle-income countries. That said, the uncontrolled intake of medicines, even herbal ones, can be harmful to health. It can lead to serious fetal-maternal consequences during pregnancy. It is therefore important to scientifically assess the limits of toxicity of drugs derived from medicinal plants for empirical therapeutic use. Many studies have evaluated the toxic potential of medicinal plants on female reproductive function and embryo-fetal development (Paula et al., 2020; Abdulmannan et al., 2019). The present study was conducted to evaluate the toxic effects of *A. oligophyllus* leaves aqueous extract on motherhood, reproductive

Table 4. Hematology parameters in *A. oligophyllus* aqueous extract-treated pregnant rats.

Parameter	Groups			
	Control	**AoAE 50**	**AoAE 100**	**AoAE 200**
RBC (10^4/mm^3)	392.5 ± 66.1	434.5 ± 34.8	389.8 ± 60.6	408.2 ± 29.4
WBC (10^2/mm^3)	46.7 ± 6.7	54.5 ± 5.5	23.3 ± 1.1	43.2 ± 3.4
HGL (g/dL)	14.5 ± 0.9	13.0 ± 1.0	11.3 ± 1.7	12.1 ± 0.6
Hematocrit (%)	38.3 ± 4.5	40.9 ± 1.7	36.3 ± 3.7	39.3 ± 1.7
MGV (fl)	83.1 ± 2.8	86.8 ± 1.1	83.8 ± 0.9	86.9 ± 1.3
MCHL (pg)	28.1 ± 1.5	29.1 ± 0.9	25.7 ± 1.7	27.7 ± 1.0
MCHC (g/dl)	32.8 ± 0.8	33.8 ± 0.6	31.5 ± 1.1	32.9 ± 0.6
PLT (10^3G/L)	129.0 ± 16.1	133.4 ± 13.0	142.5 ± 18.7	134.0 ± 13.5

Values are expressed as mean ± ESM; n = 6; AoAE 50, AoAE 100 and AoAE 200 = *Angylocalyx oligophyllus* aqueous extract at doses indicated.
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Table 5. Effects of *A. oligophyllus leaves* aqueous extract in reproductive female parameters.

Parameter	Groups			
	Control	**AoAE 50**	**AoAE 100**	**AoAE 200**
Pregnant female (Day 0)	6	6	6	6
Effectively pregnant female (Day 20)	6	6	6	6
% Pregnancy	100	100	100	100
Corpora lutea (N) (Mean ± ESM)	138 (23.0 ± 1.4)	159 (26.5 ± 2.8)	149 (24.8 ± 1,7)	152 (25.3 ± 2.2)
Implantation (Mean ± ESM)	63 (10.5 ± 0,2)	68 (11.3 ± 0.7)	58 (9.7 ± 1.1)	59 (9,8 ± 0.4)
Birth index	15.1 ± 1.3	13.0 ± 2.2	15.5 ± 1.2	16.4 ± 1.5
Live fetuses (Mean ± ESM)	60 (10.0 ± 0.7)	55 (9.2 ± 1.5)	59 (9.8 ± 0.8)	55 (9.2 ± 0.7)
Dead fetuses	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Resorptions (Mean ± ESM)	5 (0.8 ± 0.4)	7 (1.2 ± 0.7)	3 (0.6 ± 0.2)	5 (0.8 ± 0.5)
Pre-implantation loss (%)	53.7 ± 2.4	62.3 ± 1,5	60.2 ± 5.1	59.9 ± 3.4
Post-implantation loss (%)	11.1 ± 4.4	21.7 ± 5.6	17.0 ± 4.6	14.1 ± 5.5
Maternal weight gain minus (g)	13.4 ± 2.1	10.3 ± 3.6	11.3 ± 2.3	10.2 ± 3.1
Gravid uterus weight (g)	20.6 ± 1.0	19.7 ± 1.3	22.6 ± 1.0	18.7 ± 1.0

Values are expressed as mean ± ESM; n = 6; AoAE 50, AoAE 100 and AoAE 200 = *Angylocalyx oligophyllus* aqueous extract at doses indicated. Data in bold represent the sum in each group.
Source: Experience Results | Generated with Office Word

Table 6. Effect of *A. oligophyllus leaves* aqueous extract in morphological parameters of fetuses.

Parameter	Groups			
	Control	**AoAE 50**	**AoAE 100**	**AoAE 200**
Fetal body weight (g)	4.0 ± 0.7	4.1 ± 0.7	4.1 ± 0.6	4.0 ± 1.0
SGA fetal (%)	10.0	5.5	5.1	20.0
AGA fetal (%)	90.0	89.1	91.5	69.1
LGA fetal (%)	0.0	5.5	3.4	10.9

Values are expressed as mean ± 1.7 × SD; n = 6; AoAE 50, AoAE 100 and AoAE 200 = *Angylocalyx oligophyllus* aqueous extract at doses indicated. SGA: Small for Gestational Age; AGA: Adequate for Gestational Age; LGA: Large for Gestational Age.
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Table 7. Effect of *A. oligophyllus leaves* aqueous extract in biochemical parameters.

Parameter	Groups			
	Control	AoAE 50	AoAE 100	AoAE 200
Blood glucose (mg/kg)	50.0 ± 2.1	53.0 ± 4.0	52.3 ± 3.5	50.2 ± 2.8
Total protein (g/dL)	1.4 ± 0.2	1.3± 0.1	1.3 ± 0.2	1.3 ± 0.2
ALT (U/L)	50.2 ± 2.2	43.8 ± 2.4	39.0 ± 4.3	50.6 ± 1.57
AST (U/L)	168.4 ± 3.0	173.1 ± 3.9	197.9 ± 8.6	178.2 ± 4.1
Serum urea (mg/dL)	2.9 ± 0.4	2.5 ± 0.2	2.4 ± 0.3	2.8 ± 0.2
Serum creatinine (mg/dL)	1.0 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Urine creatinine (mg/dL)	33.2 ± 5.7	16.1 ± 1.2 *	43.7 ± 8.1	29.9 ± 2.1

Values are expressed as mean ± ESM; n = 5, *p <0.05 = significant difference from control, AoAE 50, AoAE 100 and AoAE 200 = *Angylocalyx oligophyllus* aqueous extract at doses indicated.
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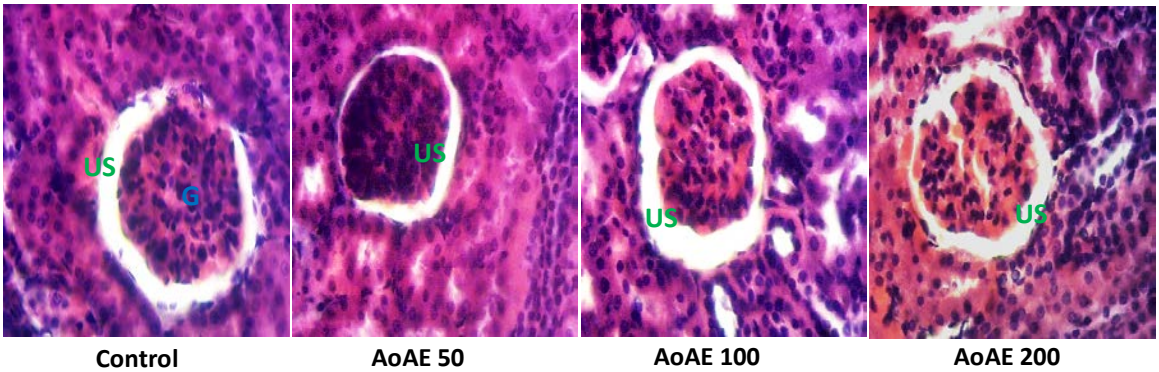

Figure 3. Kidney morphological data of rats treated with *A. oligophyllus* leaves aqueous extract during pregnancy (HE, 200X). G = Glomerule; US = Urinary space; AoAE 50, AoAE 100 and AoAE 200 = *Angylocalyx oligophyllus* aqueous extract at doses indicated.
Source: Camera equiped ligth microscope micrographs

parameters and fetal development. First of all, the acute toxicity of this plant extract was performed in non-pregnant rats. Acute administration of the *A. oligophyllus* leaves aqueous extract at a dose of 2000 mg/kg did not cause any adverse effect in non-pregnant female rats, suggesting that this plant extract could be classified as "not toxic or very slightly toxic", with the LD_{50} higher than 2000 mg/kg of body mass, and the therapeutic dose approximately 200 mg/kg body weight according to OECD guideline number 423 (OECD, 2001).

During embryonic development, maternal toxicity is one of the causes of embryonic and fetal malformations and is diagnosed in the mother by weight loss, a decrease or increase of relative organs masses, altered blood form counts and death of fetuses (Raza et al., 2002; Beyer et al., 2011). Prolonged administration of the *A. oligophyllus* leaves aqueous extract at doses of 50, 100 and 200 mg/kg in pregnant rats (from day 0 to day 20 of pregnancy) did not significantly modify the body mass increase, blood form counts and relative organ masses of treated pregnant rats, compared to pregnant control rats. However, the slight significant increase in the spleen's relative mass observed at the dose extract of 50 mg/kg would probably not be related to the plant extract, since no relative organs masses variation was observed after the acute administration of the dose of 2000 mg/kg of this extract (that is, twice the total quantity of extract ingested in 20 days of treatment by an animal receiving the dose of 50 mg/kg). Moreover, the numbers of hematological parameters (red blood cells, white blood cells and blood platelets) did not vary between the groups, confirming that the spleen's relative mass increase would not be related to the plant extract effect. These results suggest that the *A. oligophyllus* leaves aqueous extract does not affect maternity. Other plants such as *Bryophyllum pinnatum* (Hosomi et al., 2014), *Verbena officinalis* (Abdulmannan et al., 2019) and *Phyllanthus niruri* (Paula et al., 2020) have also been reported not to affect weight

gain, relative organ masses, and blood form counts when given to pregnant rats. Similarly, Grance et al. (2008) showed that the administration of *Baccharis trimera* extract did not modify the hematological parameters Fetal-maternal nutritional needs increase during pregnancy, which leads to a consequent increase in carbohydrate, protein and lipid metabolism for the maintenance of pregnancy. However, a disturbance of this metabolism at this time by exogenous or endogenous factors can lead to fatal consequences such as miscarriages, fetal malformations, etc. In addition, the affection or alteration of the organs involved in the metabolism of nutrients and toxic substances such as the liver and the kidneys (whose serum levels of transaminases, urea and creatinine provide information on the integrity and the functioning state) can also lead to harmful effects (Giboney, 2005). The serum levels of glucose, proteins, transaminases, urea and creatinine did not vary between the pregnant rats treated with the different doses of *A. oligophyllus* and the untreated pregnant rats. These results indicate that the aqueous extract of *A. oligophyllus* does not alter nutrients metabolism or liver and kidney function and integrity. The maintenance of the normal kidney tissue integrity after 20-days treatment confirms these results. Paula et al. (2020) also showed that *P. niruri* extract does not modify blood glucose levels and has hepatoprotective effect in pregnant rats. The creatinine resulting from the degradation of muscle creatine is mostly eliminated by the kidneys and its urinary level is much higher than its serum level. During pregnancy, the increase in renal blood flow is accompanied by an increase in glomerular filtration rate responsible for a drop in plasma creatinine, and consequently an increase in urinary creatinine. However, the drop observed in urinary creatinine of rats having received the extract dose of 50 mg/kg would probably be linked to compensatory hyperhydration increasing the diuresis and consequently lowering the urinary creatinine. The absence of major alteration of renal tissue confirms that this slight drop in urinary creatinine is not linked to any renal disease.

Although a substance does not have harmful effects on motherhood, it can still affect fertility. Fertility (ability to have children) is determined by the number of implantations, pre- and post-implantation losses and litter (Gerenutti et al., 2008). During implantation, the embryo can either continue its normal development or be reabsorbed (Van Mourik et al., 2009). Some substances containing molecules that mimic the action of steroid hormones (Chen et al., 2013), so can cause intrauterine growth retardation and/or embryo resorption, thus altering the fertility (Leite et al., 2004). In this study, the *A. oligophyllus* aqueous leaf extract did not modify the number of implantation sites, suggesting that the saponins contained in this extract would have improved the quality of oocytes by reducing the number of resorption sites, mainly at doses of 100 and 200 mg/kg. Likewise, the percentages of pre- and post-implantation losses did not vary significantly between the treated and untreated groups, showing that the metabolites contained in the extract do not negatively affect embryo-fetal development. The prenatal development evaluated by the weight of the fetuses showed no significant difference at day 20 of gestation between the fetuses of the *A. oligophyllus* aqueous extract treated mothers and those of control mothers, which justifies the unvaried increase in mothers' masses between these groups during pregnancy. However, the high percentage of small (20%) and large (11%) pups for the gestational age borned from the females treated with the dose extract of 200 mg/kg compared to the controls (although not significant), suggest that a margin of attention is needed as to the dose of extract to be taken during pregnancy. Paula et al. (2020) also observed that the high dose of *P. niruri* (600 mg/kg) affects fetal development more than the low doses (150 and 300 mg/kg).

Alkaloids are a special class of natural organic compounds synthesized as secondary metabolites showing strong biological activities in very small doses, but also many harmful effects in high doses on animal and human organisms. Thus, the absence or low toxicity of the aqueous extract of *A. oligophyllus* would be linked not only to the presence of compounds with beneficial effects such as flavonoids, polyphenols, tannins, sterols, etc., but also and very probably to the low content of alkaloids contained in this plant extract.

Conclusion

The present data show that the *A. oligophyllus* leaves aqueous extract does not impair motherhood (maternal parameters) and reproduction. Nevertheless, limitation of the dose is recommended during treatment in pregnant women to avoid adverse effect on fetal development.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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