



Reproductive Characteristics, Serum Metabolites, and Oxidative Status in Female Guinea Pigs (*Cavia porcellus*) Fed with Ethanolic Extract of *Dichrostachys glomerata* Fruit

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ABSTRACT

Dichrostachys glomerata (*D. glomerata*) is an aromatic plant which is used as a spice in cooking and Cameroonian traditional medicine to treat infertility in men. This work was designed to highlight the effects of the ethanolic extract of *D. glomerata* on oxidative status, serum metabolites and reproductive characteristics in female guinea pigs (*Cavia porcellus*). A total of 48 primiparous female guinea pigs, aged 4 months old with the body weight of 400 ± 10 g, were divided into four groups with two replications per group (6 guinea pigs each). During 90 days of trial, Group 1 (control group) orally received 1 ml/kg b.w. of distilled water daily, and groups 2, 3, and 4 received *D. glomerata* ethanolic extract once a day at doses of 50, 100, and 200 mg/kg b.w. using the same method of administration, respectively, for 90 days, including 60 days of gestation. After the first 30 days of treatment, mating was done by placing one non-treated male into cages containing six treated females. At the end of the treatment, data were collected on reproductive characteristics, serum metabolites, and oxidative stress markers. The results revealed that the ethanolic extract of *D. glomerata* induced a significant decrease in the number of post-implantation resorption and ovaries weight. Groups 3 and 4 showed a significant increase in the number of fetuses per dam and viable fetuses as well as placenta weight, compared to the control group. The serum level of progesterone significantly decreased in the group treated with 200 mg/kg *D. Glomerata*, compared to the other treated groups. The extract at 100 mg/kg body weight showed a significant increase in fetuses weight and fetuses crown-rump length, compared to the control group. Catalase activity significantly increased in the control group than *D. glomerata* treated groups. In conclusion, ethanolic extract of *D. glomerata* minimized reproductive stress and subsequently improved the reproductive performance of guinea pigs.

Keywords: *Dichrostachys glomerata*, Guinea Pig, Oxidative Stress, Reproduction, Serum Metabolites

INTRODUCTION

Guinea pigs (*Cavia porcellus*) cannot produce their own vitamin C (Michel et al., 2011) that acts as an antioxidant in the body under oxidative stress. Environmental factors, such as pollution, climatic change, poor quality of feed and water, as well as diseases are associated with oxidative stress that is responsible for low reproductive performance (Ngoula et al., 2020), as well as high morbidity and mortality. In addition, Deutcheu et al. (2020) observed a decrease in the fertility rate, fetal weight, viability, ovaries, and uterus weight in adult female guinea pigs exposed to oxidative stress for 90 consecutive days. In order to neutralize the environmental effects and increase animal productivity, antibiotics were massively used as feed additives. Unfortunately, antibiotic resistance was developed in pathogenic microbiota and its use was banned by the European Union and the authorities of many countries in the world (Alleman et al., 2013).

As a solution, attention has been focused on natural plant by-products rich in molecules with antioxidant properties (El-kaiaty et al., 2020). In Africa, especially in the tropical region, a larger number of plants and their extracts possess various pharmacological activities, including antioxidant, anti-inflammatory, anti-cancer, anti-microbial, and aphrodisiac properties (Raji et al., 2006). Among those tropical plants, *Dichrostachys glomerata* (*D. glomerata*), an aromatic plant from the family Fabaceae, is used worldwide as a spice and medicinal drug to cure infertility in men (Tchiégang et al., 2005). The study on the chemical components of *D. glomerata* fruit showed that it is rich in flavonoids, phenolic compounds, alkaloids, tannins, saponins, and terpenoids. These molecules possess numerous pharmacological activities, including cardioprotective, anti-inflammatory, antimicrobial, antioxidant, and hepatoprotective properties (Kuate et al., 2010; Kothari et al., 2010; Fankam et al., 2011). Due to these activities, *D. glomerata* can be used in animal production to limit the negative effects of environmental factors, and subsequently improve growth and reproductive performances.

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The aim of the current study was to investigate the effects of ethanolic extract of *D. glomerata* on reproductive parameters, serum metabolites, and oxidative stress markers in female guinea pigs.

MATERIALS AND METHODS

Ethical approval

Experimental protocols used in this study were approved by the ethics committee of the Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon, and strictly conformed with the internationally accepted standard ethical guidelines for laboratory animal use and care described in the European Community guidelines; EEC Directive 86/609/EEC, of November 1986.

Plant source and preparation of the extract

Dry fruits of *D. glomerata* were bought from a local market of Bafoussam, West Region of Cameroon. They were then ground into a fine powder, conserved in an opaque flask. Following that ethanolic extract was prepared using the procedure described by Yakubu et al. (2005). Briefly, 500 g of *D. glomerata* powder was macerated in ethanol 70% for 72 h at room temperature. The extract was filtered with Whatman paper No. 3 and the resulting filtrate was concentrated by rotatory evaporation to remove ethanol, and further lyophilized to obtain a fine powder. The phytochemical screening of *D. glomerata* extract was done as described by Ramde-Tiendrebeogo et al. (2012) and Padmaja et al. (2011).

Experimental animals

The study was conducted from February to May 2019 at the Teaching and Research Farm of University of Dschang, Cameroon. A total of 48 four-month-old female guinea pigs with an average body weight of 400 ± 10 g were obtained from the Teaching and Research Farm of the University of Dschang, Cameroon. Moreover, eight adult males were used only as sires and were not treated. Throughout the experimental period (90 days), drinking water and commercial complete feed Société des Proviendries du Cameroun SA containing 16% crude protein, 2350 kcal/kg metabolizable energy, and 7% crude fiber were offered *ad libitum*.

Experimental design

Before starting the experiment, the animals were weighed and identified using numbered ear tags. In a completely randomized design, the female guinea pigs were divided into four equal groups (12 animals each). Each group was replicated two times with six females per replicate with comparable average body weight. The samples in the first group, which was the control group (T0) orally received 1 ml/kg bw distilled water. Groups 2, 3, and 4 received *D. glomerata* ethanolic extract once a day at doses of 50, 100, and 200 mg/kg b.w., respectively. All treatments were administered directly into the mouth using 5-ml syringe and daily for 90 days, including 60 days of pregnancy. After the first 30 days of treatment, mating was done by placing one non-treated male into cages containing six treated females. After 60 days of gestation, six animals were selected at random for sacrifice to investigate fetus characteristics.

Organ collection and fetal parameters assessment

At the end of the trial (day 90), six animals from each group were anesthetized using ethyl ether. The blood was collected from the jugular vein and stored at room temperature for biochemical analyses. Fetuses and organs (liver and ovaries) were carefully removed, separated from fat residues, and weighed separately using a scale of 160 g capacity and 10^{-3} g precision. The number of corpus luteum per ovary was directly naked-eyed counted. The number of pre-and post-implantation resorption was calculated by using formulas proposed by Waalkens-berendsen et al. (1998), as follows:

Pre-implantation resorptions = number of corpus luteum – number of implantation site

Post implantation resorptions = number of implantation sites – number of viable fetuses.

The lengths of the fetus, head, and rump were measured using an electronic caliper. The number and weight of fetuses, viability, and fetal mortality rate were also measured. One part of the liver was randomly selected from each treatment, sliced and crushed in a known volume of cold NaCl, 0.9% to obtain a 15% homogenate. The resulting homogenate was centrifuged at 3000 rpm for 30 min at -0°C , and aliquot supernatants were used for oxidative stress markers analyses.

Progesterone and biochemical parameters quantification

Serum progesterone level was determined using appropriate commercial ELISA kits (Omega diagnostics kit Scotland, United Kingdom). Toxicity markers, such as the levels of total proteins, total cholesterol, creatinine, urea, AST, and ALT, were determined using the CHRONOLAB kit (Barcelona, Spain) following the manufacturer's protocol. Oxidative stress markers, including superoxide dismutase (SOD), catalase (CAT), total peroxidises (POX) activities,

malondialdehyde (MDA), and nitric oxide (NO) concentrations, were measured using the spectrophotometer (GENESYS 20.0, England) and according to the methods described by Misra and Fridovich. (1972), Sinha. (1972), Habbu et al. (2008), Nilsson et al. (1989), and Giustarini et al. (2008), respectively.

Statistical analysis

Statistical analyses were performed using SPSS software (IBM SPSS, USA, version 20). The difference between treatments was assessed using one-way ANOVA followed by Duncan's post hoc test. The limit of significance was 5% and the results were expressed as mean \pm standard deviation.

RESULTS

Phytochemical constituents

Results of phytochemical screening of ethanolic extract of *D. glomerata* are summarized in Table 1.

Effects of ethanolic extract of *Dichrostachys glomerata* on fetus toxicity parameters

Fetuses toxicity parameters in guinea pigs treated with ethanolic extract of *D. glomerata* are shown in Table 2. The numbers of corpus luteum per dam, implantation site, fetuses per dam, viable fetuses per dam, of placenta and placenta weight insignificantly increased with the higher doses (100 and 200 mg/kg b.w.) of ethanolic extract of *D. Glomerata*, compared to the control ($p > 0.05$). The relative weight of ovaries was significantly decreased with the increased doses of ethanolic extract of *D. Glomerat* when compared to that of animals in the control group ($p < 0.05$). The number of post-implantation resorptions per dam significantly increased with the smallest dose (50 mg/kg b.w.) of *D. glomerata* extract, compared to the control group ($p < 0.05$). The number of dead fetuses per dam was comparable between female treated with 50 mg/kg b.w and those receiving distilled water ($p > 0.05$) while no dead fetus and post-implantation resorption were registered at the higher doses of *D. glomerata* extract (100 and 200 mg/kg b.w.). Regardless of the treatment, no pre-implantation resorption was recorded.

Effects of ethanolic extract of *Dichrostachys glomeration* on fetus growth characteristics

As can be seen in Table 3, fetus weight significantly increased in the females treated with 100 mg/kg b.w. of *D. glomerata* ethanolic extract, compared to animals of other treatments ($p < 0.05$). Regardless of females in the control group who had a comparable head length to those received 100 mg/kg b.w. of *D. glomerata* ethanolic extract ($p > 0.05$), foetus, head, and rump lengths recorded in guinea pigs administered with 100 of *D. glomerata* ethanolic extract significantly increased, compared to those received 50 mg/kg b.w. of *D. glomerata* ethanolic extract and control group ($p < 0.05$). However, the obtained results were similar to those received 200 mg/kg b.w. of *D. glomerata* ethanolic extract ($p > 0.05$).

Effects of *Dichrostachys glomerata* ethanolic extract on serum level of progesterone in guinea pig

As shown in Figure 1, the serum level of progesterone decreased significantly in 200 mg/kg b.w. of *D. glomerata* treated group in comparison with the control and other treated groups ($p < 0.05$). Nevertheless, the serum level in progesterone recorded in the control group was comparable to those in *D. glomerata* treated animals at doses of 50 and 100 mg/kg b.w. ($p > 0.05$).

Effects of *Dichrostachys glomerata* ethanolic extract on biochemical parameters in female guinea pigs

As shown in Table 4, *D. glomerata* ethanolic extract did not significantly affect serum content in total proteins, albumin, globulins, ALT, and creatinine concentrations, compared to the control ($p > 0.05$). In guinea pigs treated with ethanolic extract of *D. glomerata*, the serum level of total cholesterol and AST significantly increased with the increasing doses of extract ($p < 0.05$). Compared to the control group the serum level of total cholesterol significantly increased only in females treated with 200 mg/kg b.w. of *D. glomerata* ethanolic extract ($p < 0.05$) and AST was comparable among all treatments ($p > 0.05$). The *D. glomerata* ethanolic extract increased the serum level of urea but, this effect was significant only at 200 mg/kg b.w., compared to the control ($p < 0.05$).

Effects of *Dichrostachys glomerata* ethanolic extract on oxidative status in female guinea pig

The results presented in Table 5 indicated that *D. glomerata* ethanolic extract doses did not significantly affect SOD, total peroxidase activities, MDA, and NO concentrations ($p > 0.05$). Although statistically comparable, the level of MDA and NO decreased with *D. glomerata* at doses of 100 and 200 mg/kg b.w., compared to control. The activity of catalase was comparable in *D. glomerata* treated guinea pigs, but significantly lower, compared to the value recorded in control animals ($p < 0.05$).

Table 1. Phytochemical constituents of *Dichrostachys glomerata* ethanolic extract.

Constituents	
Flavonoids	+
Alkaloids	+
Phenols	+
Tannins	+
Triterpenes	+
Steroids	-
Saponins	+

+ : Presence of phytochemicals, - : Absence of phytochemical.

Table 2. Ovary weight and fetus index in female guinea pigs treated with ethanolic extract of *Dichrostachys glomerata*

Foetotoxicity characteristic	Doses of <i>Dichrostachys glomerata</i> extract (mg/kg b.w.)				p-value
	0 (n = 6)	50 (n = 6)	100 (n = 6)	200 (n = 6)	
Number of corpus luteum/dam	2.33±0.52	2.67±0.52	2.50±0.55	2.50±0.55	0.684
Relative weight of both ovaries (g/100 g b.w.)	0.009±0.008 ^a	0.008±0.001 ^{ab}	0.007±0.001 ^b	0.007±0.002 ^b	0.048
Number of implantation sites/animal	2.33±0.52 ^b	2.67±0.52 ^a	2.50±0.55 ^{ab}	2.50±0.55 ^{ab}	0.048
Number of pre-implantation resorptions/animal	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.000
Number of post-implantation resorptions/ animal	0.17±0.05 ^b	0.33±0.12 ^a	0.00±0.00	0.00±0.00	0.049
Number of fetus/dam	2.33±0.52 ^{ab}	2.33±0.52 ^{ab}	2.50±0.55 ^a	2.50±0.55 ^a	0.046
Number of viable fetuses/ dam	2.17±0.41 ^{ab}	2.00±0.52 ^b	2.50±0.55 ^a	2.50±0.55 ^a	0.049
Number of dead fetuses/ dam	0.17±0.05	0.33±0.12	0.00±0.00	0.00±0.00	0.596
Number of placenta/dam	2.33±0.52	2.33±0.52	2.50±0.55	2.50±0.55	0.083
weight of placenta (g)	4.25±0.71 ^{ab}	3.63±0.74 ^b	4.49±0.45 ^a	4.51±0.49 ^a	0.025

^{a, b}: In the same row, means with different letters are significantly different (p < 0.05). n: Number of animals

Table 3. Effects of ethanolic extract of *Dichrostachys glomerata* on fetus growth characteristics

Fetuses mensuration	Doses of <i>Dichrostachys glomerata</i> extract (mg/kg b.w)				p value
	0 (n = 6)	50 (n = 6)	100 (n = 6)	200 (n = 6)	
Fetus weight (g)	41.18±7.89 ^{cb}	39.69±4.45 ^c	55.64±6.09 ^a	47.75±5.61 ^b	0.001
Fetus length (mm)	8.58±0.94 ^b	8.84±1.13 ^b	10.06±1.18 ^a	9.39±0.52 ^{ab}	0.000
Head length (mm)	3.28±0.26 ^{ab}	3.13±0.51 ^b	3.58±0.41 ^a	3.44±0.44 ^{ab}	0.047
Rump length (mm)	1.97±0.29 ^b	2.09±0.35 ^b	2.54±0.41 ^a	2.53±0.44 ^a	0.001

^{a, b, c}: In the same row, means with different letters are significantly different (p < 0.05). n: number of animals.

Table 4. Effects of ethanolic extract of *Dichrostachys glomerata* on biochemical parameters in female guinea pig

Biochemical parameters	Doses of <i>Dichrostachys glomerata</i> extract (mg/kg b.w)				p value
	0 (n = 6)	50 (n = 6)	100 (n = 6)	200 (n = 6)	
Serum total proteins (g/dl)	6.74±1.10	5.90±0.61	6.69±0.81	6.83±0.56	0.363
Albumin (g/dl)	3.33±0.61	2.79±0.29	2.74±0.64	3.00±0.76	0.518
Globulins (g/dl)	3.41±0.78	3.11±0.57	3.95±0.76	3.83±0.46	0.293
Total cholesterol (mg/dl)	48.83±9.1 ^{bc}	38.76±7.37 ^c	54.38±4.0 ^b	65.70±6.14 ^a	0.003
ALT (UI)	55.37±9.08	42.00±7.58	46.38±6.86	49.06±9.32	0.169
AST (UI)	51.19±6.96 ^{ab}	44.63±5.80 ^b	59.00±7.73 ^a	57.44±9.15 ^a	0.041
Creatinine (mg/dl)	1.48±0.27	1.18±0.25	1.28±0.29	1.24±0.25	0.360
Urea (mg/dl)	42.24±7.52 ^b	39.58±6.54 ^b	48.95±4.35 ^{ab}	58.13±6.91 ^a	0.006

^{a, b, c}: In the same row, means with different letters are significantly different (p < 0.05). n: Number of animals. ALT: Alanine transaminase; AST: Aspartate transaminase

Table 5. Oxidative stress markers in guinea pig treated with ethanolic extract of *Dichrostachys glomerata*

Oxidative stress markers	Doses of <i>Dichrostachys glomerata</i> extract (mg/kg b.w)				p value
	0 (n = 6)	50 (n = 6)	100 (n = 6)	200 (n = 6)	
SOD (U/min/g of hepatic proteins)	0.35±0.07	0.39±0.05	0.43±0.05	0.39±0.09	0.334
CAT (µM/min/g of hepatic proteins)	1.67±0.26 ^a	1.28±0.10 ^b	1.19±0.14 ^b	1.04±0.19 ^b	0.000
Total peroxidase (mM/min/ g of hepatic proteins)	30.58±7.38	28.33±4.69	24.28±2.17	29.88±2.51	0.267
MDA (µM/g of liver)	2.55±0.45	2.26±0.41	2.11±0.34	2.09±0.29	0.259
NO (µM/g of liver)	21.00±3.97	21.23±4.50	17.47±2.35	17.49±3.62	0.334

^{a, b}: In the same row, means with different letters are significantly different (p < 0.05). n: Number of animals.

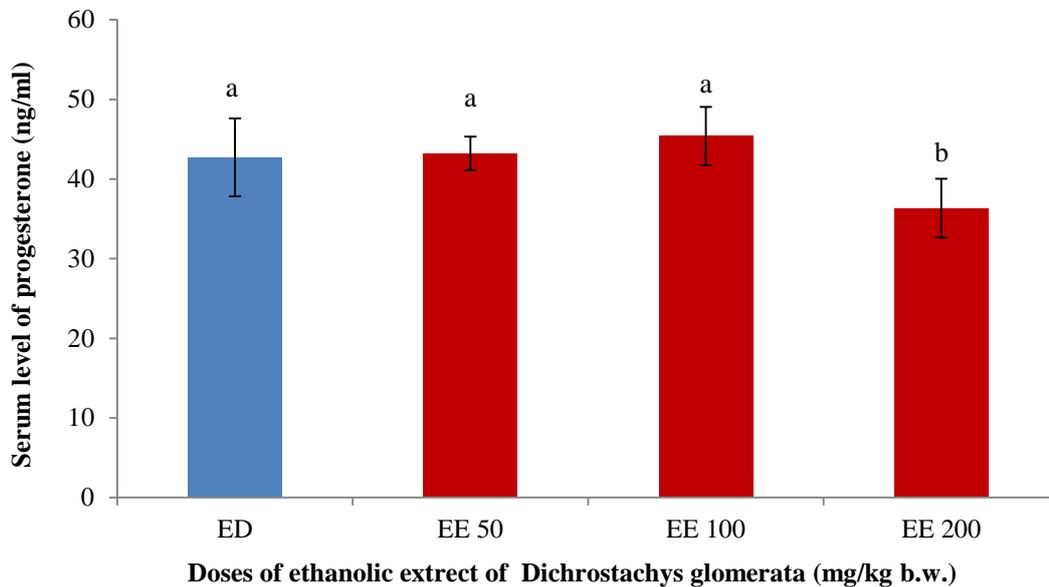


Figure 1. Effects of ethanolic extract of *Dichrostachys glomerata* on serum level of progesterone in guinea pigs treated with different doses. ^{a, b}: Means with different letters are significantly different ($p < 0.05$). ED: Distill water. EE 50, EE 100, and EE 200: 50, 100, 200 mg/kg b.w of *D. glomerata* ethanolic extract, respectively.

DISCUSSION

The chemical screening of *D. glomerata* ethanolic extract carried out in the present study revealed the presence of phenols, tannins, triterpenes, saponins, alkaloids, and flavonoids. These molecules have diverse activities (antioxidants, antibacterial, anti-inflammatory, antiseptic, antiparasitic, and immunomodulatory properties) and can improve animal reproductive characteristics (Kuate et al., 2010; Tchoffo et al., 2019).

Oral administration of *D. glomerata* ethanolic fruits extract at doses of 100 and 200 mg/kg b.w. for 90 consecutive days to female guinea pig increased the number of implantation sites, fetuses per dam, and viable fetuses. These results corroborated those obtained by Watcho et al. (2009) who observed a significant increase in the number of implantation sites and the number of litter sizes per dam in rats treated during 7 consecutive days with 100 mg/kg b.w. of aqueous extract of *Ficus asperifolia*. These results are related to the bioactive molecules present in *D. glomerata* fruit which can induce a favorable milieu for zygote implantation and development. According to Grzanna et al. (2005) phenolic and alkaloids compounds present in *D. glomerata* have reputed to reinforce the endogenic antioxidant system of animals and protect embryonic tissue against reactive oxidative stress attacks. This effect subsequently enhances zygote implantation, fertility rate, and the survival rate of animals after birth.

Progesterone is required in all mammals to support the survival and development of the embryo/fetuses and attached membranes (Spencer and Bazer, 2002). It is produced by the ovaries and placenta and helps to prepare the body for conception and pregnancy (Montaserti et al., 2007). The results of the present study showed an increased level of progesterone in female groups treated with 50 and 100 mg/kg b.w. of ethanolic extract of *D. glomerata*. These results agreed with those obtained by Bafor et al. (2015) in mice received orally *Alchornea laxiflora* methanol leaf extract at 1000 mg/kg for 6 consecutive days and Yakubu et al. (2008) in female rats treated with *Cnidioscolous aconitifolius* for 7 consecutive days. This observation can be attributed to some bioactive molecules, such as alkaloids and phenols, present in *D. glomerata* extract that protect corpus luteum and placenta from reactive oxygen species attacks, and subsequently favor the growth and function of the cells.

In the present study, all fetuses' growth characteristics (fetus weight, fetus, head, and rump length) significantly increased in animals treated with 100 mg/kg of *D. glomerata* ethanolic extract, compared to the control group. This effect can be due to the best plasmatic availability and mobilization of nutriment for fetuses' growth during gestation. This hypothesis is supported by the increase in serum level of total proteins, albumin, globulins, and total cholesterol with a high dose of *D. glomerata* extract. These results are in agreement with those obtained by Ebile et al. (2018), who observed an increased level of total proteins, albumin, globulins, and total cholesterol in quails treated with 5 g of *D. glomerata* powder incorporated in drinking water.

The decreased level of ALT observed in the present study agreed with those recorded by Kothari et al. (2014) in rats treated orally with 1000 mg/kg of *D. glomerata* fruit ethanolic extract for 90 days. This observation can be directly related to the hepatoprotective effects of ethanolic extract of *D. glomerata*. However, the increased level of AST can be due to the fact that AST is produced by many other organs, such as kidney, heart, and muscle, apart from the liver.

Additionally, the significantly increased level of urea recorded in the current study was positively correlated with higher kidney metabolism and consequently responsible for AST increased level. These results disagreed with those observed by Kothari *et al.* (2014) and Ebile *et al.* (2018). Urea and creatinine are used to evaluate the level of protein metabolism and kidney function. The present study indicated an increased level of urea and a decreased level of creatinine which was in contrast with findings of Kothari *et al.* (2014) and Ebile *et al.* (2018). The obtained results of the present study resulted from the increased level of protein metabolism which could make some changes in kidney cell function.

Pregnancy is a state characterized by an increase in reactive oxygen species (ROS) production, particularly placental metabolic and steroidogenic activities involved in the increase of oxygen consumption by the fetoplacental unit (Myatt and Cui, 2004). In the current study, there was an increase in SOD as the first antioxidant enzyme to neutralize the ROS (Agarwal *et al.*, 2005). Meanwhile, the decreased level of MDA and NO levels can express the cell-protective effects of *D. glomerata* extract. These results agree with those obtained by Kuate *et al.* (2010) who analyzed the *in vitro* antioxidant activity of *D. glomerata* extracts. In fact, the phytochemical screening of *D. glomerata* extract revealed the presence of alkaloids, saponins, phenolics, tannins, flavonoids, and triterpenes which have been recognized to have antioxidant properties (Sen *et al.*, 2010).

CONCLUSION

The present study demonstrated the oral administration of *D. glomerata* ethanolic extract at doses of 100 and 200 mg/kg b.w. positively influenced the fetuses growth characteristics. Due to the diverse bioactive molecules with antioxidant properties, *D. glomerata* ethanolic extract protects the animal cells, including reproductive cells from reactive oxygen species attacks which subsequently improves animal reproductive performances.

DECLARATIONS

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Authors' contributions

Ngoula Ferdinand and Kouamo Justin supervised and designed the project, cross-checked the draft of the manuscript, and finally approved it for submission. Djuissi Motchewo Nadège and Ngoula Ferdinand designed the project, conducted the experiment, analyzed data, and wrote the first draft of the manuscript. Nono Fambo Stive Mathieu and Lontio Fulbert Aimé assisted in the conduction of the experiment and data collection. Vemo Bertin Narcisse, Tchoffo hervé, and Dongmo Nguedia Arius conducted laboratory analyses of the experiment. All the authors approved the final draft of the manuscript for submission.

Competing interests

Authors have declared that no competing interests exist.

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