



## Effects of Aqueous and Ethanolic Extracts of Ginger (*Zingiber Officinale*) Rhizome on Serum Progesterone Level and Markers of Oxidative Stress in African Giant Rat (*Cricetomys Gambianus*) in Captivity

Foda F.C.<sup>1\*</sup>, Mingoas K.J.P.<sup>2</sup>, Chongsi M.M.M.<sup>1</sup>, Fonou T.L.<sup>3</sup>, Takam B.B.I.<sup>1</sup>,  
Fonkem S.<sup>1</sup>, Vemo B.N.<sup>3</sup> and Kenfack A.<sup>1</sup>

<sup>1</sup>Department of Animal Science, Faculty of Agronomy and Agricultural Science, University of Dschang, Cameroon

<sup>2</sup>Department of Physiology and Biochemistry, School of Sciences and Veterinary Medicine, University of Ngaoundéré, Cameroon

<sup>3</sup>Department of Animal Science, Faculty of Agriculture and Veterinary Medicine, University of Buea, Cameroon

\*Corresponding Author: Foda F.C., E-Mail: [fodafopaconstant@gmail.com](mailto:fodafopaconstant@gmail.com)

### ABSTRACT

The African giant rat is a very prolific animal in its natural biotope. But in captivity, this species exhibits poor reproductive performance due to embryonic resorption. These resorptions are due to oxidative stress or a lack of progesterone production. This study was carried out to evaluate whether aqueous and ethanolic extracts of ginger, given their antioxidant and steroidogenic properties, can improve the antioxidant status and serum progesterone level during gestation in African giant rats. For this purpose, twenty-eight adult female African giant rats were divided into seven treatments comparable in terms of body weight: the control group received only avocado paste (7 g/day), while the other groups received either aqueous or ethanolic extracts at doses of 250, 500, and 750 mg/kg bw in a homogeneous mixture of paste. Animals were individually housed and had free access to food and drinking water. Eight days after the start of the test, the females were bred with males. Each female was sacrificed on the 15th day post-coitus, then the blood was collected from the jugular vein and centrifuged at 3500 rpm for 15 minutes and the serum obtained was used for the determination of progesterone and markers of oxidative stress concentrations. The results showed that except for females who had received 750 mg/kg bw of aqueous extract, the serum progesterone level was significantly ( $P<0.05$ ) higher in the treated animals compared to the control. The administration of the ethanolic extract at doses of 250 and 500 mg/kg bw induced a significant ( $P<0.05$ ) increase in the levels of malondialdehyde, catalase and superoxide dismutase compared to the control group. On the other hand, the administration of aqueous extract significantly ( $p<0.05$ ) lowered the level of total peroxidases compared to the control. Aqueous and ethanolic extracts of ginger can be used to increase serum progesterone levels, but cannot be used to improve oxidative stress status.

**Keywords:** African giant rat, Ginger, Oxidative stress, Progesterone, Reproduction.

Original Article:

Received

Accepted

Published in

*J. Appl. Vet. Sci.*, 9(3): Proof

### INTRODUCTION

Despite the high reproductive potential of the African giant rat in the wild, its breeding in captivity has so far been characterized by poor reproductive performance. Most pregnancies in African giant rats are linked to embryonic resorption problems. **Fonkem et al., (2022)** reported a pre-implantation resorption rate of 47.29% and a post-implantation resorption rate of 58.33%. Those authors thought the cause might be a

defect in progesterone production. Subsequently, by treating animals with synthetic progesterone (17- $\alpha$ -hydroxyprogesterone caproate), a reduction in pre- and post-implantation resorptions to 9.82% and 13.75%, respectively, was obtained. However, it has also been documented that oxidative stress is one of the causes of embryonic resorption (**Gupta et al., 2007; Kais et al., 2010; Mutinati et al., 2013**). Synthetic products used in animal production are expensive, often unavailable, and could have more side effects; which encourages

populations to use medicinal plants such as ginger (Morel, 2008; Aouadi and Saklem, 2012).

Several studies have revealed that ginger rhizomes contain terpenes, alkaloids, phenolic compounds such as gingerdiol, gingerol, gingerdione shogaols, iron, magnesium, calcium and vitamin C (Bakkali et al., 2008; Zhao et al., 2011). These molecules are responsible for various biological activities, including antioxidant (Tapsell et al., 2006; Cécile, 2011; Iranloye et al., 2011) and androgenic (Jalil et al., 2016; Gholami-Ahangaran et al., 2021) properties. Busman and Kanedi (2016) previously showed that an aqueous extract of red ginger, administered orally, significantly increased steroidogenic hormone levels in Wistar rats. Nafiye (2018) reported a significant improvement in embryonic implantation in Wistar rats, after force-feeding them with ginger powder (100 mg/kg/day and 200 mg/kg/day) for 5 and 10 days.

In addition, the Zingiberaceae family includes plants such as ginger and turmeric, among others, which implies that these plants could share certain common characteristics. Therefore, since the work of Sirotkin et al., (2018) demonstrated that the ovaries of rabbits treated *in-vitro* with *Curcuma longa* powder released more progesterone, it could be thought that ginger could also stimulate the production of progesterone. In addition, following the treatment of diabetic Wistar rats with ginger, the malondialdehyde level drops (Shanmugam et al., 2011).

The objective of this work is to see whether the administration of aqueous and ethanolic extracts of ginger lowers the level of oxidative stress and increases the blood concentration of progesterone in African giant rats. Male or female?

## MATERIALS AND METHODS

### Study site

The study was carried out at the Teaching and Research Farm (TRF) and in the Animal Physiology and Health Laboratory of the University of Dschang, West, Cameroon.

### Animals and housing

The animal material consisted of twenty-eight adult female African giant rats (*Cricetomys gambianus*), with body weight varying from 756 to 1279 g. They were produced at the farm. Animals were housed individually in concrete boxes measuring 190 cm x 40 cm x 45 cm (length x width x height) where light was naturally provided (12 hours a day). The cages were equipped with a feed trough, a water trough and dry banana leaves used as bedding.

### Feeding

During the experiment, the animals had free access to drinking water and feed. The ratio consisted of food resources commonly consumed in the wild including corn, sweet potato, ripe banana to which the provender was added.

### Preparation of ginger extracts

Fresh ginger (*Zingiber officinale*) rhizomes were purchased from growers in a local market. These rhizomes were then washed, cut into small pieces, and dried under the sun. The dry material obtained was crushed in a mill to obtain powder. The aqueous and ethanolic extracts of ginger rhizome powder were prepared according to the methods described by Musa et al., (2006). The extracts thus obtained were introduced into opaque bottles and stored at 4°C until use.

### Extraction yield

After extracting the powder from the ginger rhizome, the extraction yield were calculated using the following formula:

$$\text{Extraction yield (\%)} = \frac{\text{Quantity of extract (g)}}{\text{Quantity of ginger powder (g)}} \times 100$$

The results obtained were as follows: 7.23% for the aqueous extract and 5.148% for the ethanolic extract. A chemical test of ginger extracts yielded the compounds shown in Table 1.

Table 1: Chemical composition of ginger extracts

Extracts	Alkaloïds	Phenols	Flavonoïds	Sterols	Triterpénoids	Tannins	Saponins	Anthocyanins	Anthraquinones
Aqueous	+	+	+	-	-	+	+	+	+
Ethanolic	+	+	+	+	+	+	+	+	+

## Experimental animals

The twenty-eight adult female African giant rats previously presented were divided into seven groups. Except for the control group that received only avocado paste (7 g/day), the other groups also received either aqueous or ethanolic extracts at doses of 250, 500 and 750 mg/kg bw in a homogeneous mixture. Eight days after the start of the test, female rats were transferred to males' compartments and followed until mating. The highlighting of the mating was done by microscopic search of spermatozoa in the vaginal mucus. Each mated female was isolated and sacrificed on the 15th day post-coitus, then the blood collected from the jugular vein was centrifuged and the serum obtained was used for the measurement of progesterone and oxidative stress markers.

## Data collection

### Progesterone and oxidative stress marker concentrations

After sacrifice of each female, the blood was collected in a test tube without anticoagulant. It was then centrifuged at 3000 rpm for 15 minutes. The serum was collected and stored at -20°C in labeled Eppendorf micro tubes for subsequent determination of progesterone and oxidative stress indicators concentrations.

The progesterone level was measured using the ELISA kit from Fortress Diagnostics Limited (produced in the United Kingdom, [www.fortressdiagnostic.com](http://www.fortressdiagnostic.com)) and manufactured to ISO 13485. The assay was carried out by the solid phase enzyme-linked immunosorbent method (ELISA) as described in the kit notice. Progesterone concentration were determined by projecting the optical densities read from the ELISA counter on to the calibration curve.

Serum concentrations of superoxide dismutase, catalase, malondialdehyde and total peroxidase were determined following the methodology described by **Misra and Fridovich (1972)**, **Sinha (1972)**, **Nilsson et al., (1989)** and **Habbu et al., (2008)**, respectively.

### Superoxide dismutase (Misra and Fridovich, 1972)

One hundred and forty (140) microliters of serum was introduced into the spectrophotometer tanks as well as 1660 µl of carbonate buffer (pH = 10) and 200 µl of adrenaline (0.3 mM). The absorbance of the adrenochrome formed was read at 480 nm 30 and 90 s after initiation of the reaction. The percentage of inhibition (I) was calculated as follows:

$$\% I = ((OD \text{ sample}) / (OD \text{ white})) \times 100$$

50% inhibition corresponding to one unit, the activity of SOD was expressed in units per quantity of proteins according to the formula opposite:

$$A = I / (50 \times \text{Protein Level})$$

### Catalase (Sinha, 1972)

To 50 µl of serum was added in test tubes 500 µl of phosphate buffer (C: 0.01 M; pH: 7). Then, 200 µl of H<sub>2</sub>O<sub>2</sub> (C: 0.2 M) was introduced into the different tubes. One minute later, 1 ml of the 5% potassium dichromate - 1% glacial acetic acid mixture (1:3) was added to the reaction medium. The whole was incubated in a boiling water bath for 10 min. After cooling the tubes with tap water, the optical density was read at 570 nm. The enzymatic activity of catalase was deduced by the Beer-Lambert law (**Servais, 2004**).

$$DO = \epsilon LC$$

**DO**: optical density read with a spectrophotometer;

**ε**: molar extinction coefficient of catalase (40 M-1cm-1);

**L**: width of the measuring tank in cm.

### Malondialdehyde (Nilsson, 1989)

500 µl of a 1% orthophosphoric acid solution and 500 µl and a mixture of 1% thiobarbituric acid in a 1% acetic acid solution were added to 100 µl of serum (Table 17). The mixture from each tube was homogenized by vortexing and incubated in a boiling water bath for 15 minutes. The tubes were then cooled in an ice bath and the mixture was centrifuged at 3500 rpm for 10 min. The absorbance of the supernatant was read at 532 nm against the control.

### Total peroxidase (Habbu et al., 2008)

To 0.5 ml of homogenate was added to 1 ml of a solution of potassium iodide KI (10 mM), then to this mixture was added 1 ml of sodium acetate (40 mM). The absorbance of potassium iodide was read at 353 nm, which indicated the amount of peroxidase. Then 20 µl of H<sub>2</sub>O<sub>2</sub> (15 mM) was added to the mixture obtained and the variation in absorbance over 5 min recorded. Units of peroxidase activity were expressed as the amount of enzyme required to change the optical density by one unit per minute. The enzymatic activity of peroxidase was deduced by the Beer-Lambert law (**Servais, 2004**) as follows:

$$O.D = \epsilon.C.L$$

With

**OD**: = optical density

**ε** = molar extinction coefficient of peroxidases (11.3 M-1cm-1)

**C** = Concentration of total peroxidases

**L** = Optical path length (1 cm).

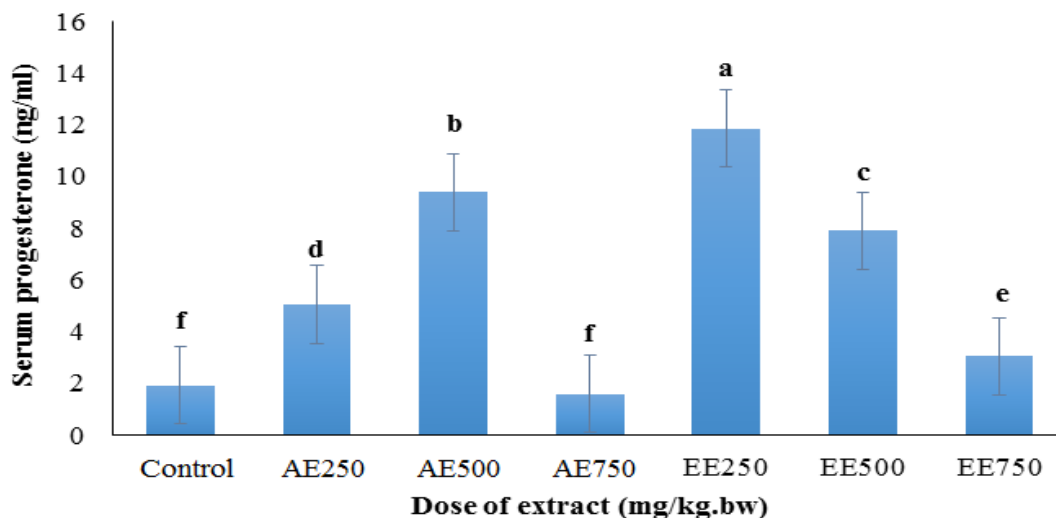
### Statistical analysis

The collected data were subjected to a one-way analysis of variance (ANOVA) to test the effect of aqueous and ethanolic extracts of ginger on the characteristics studied. Duncan test was used to separate means when there was a significant difference. Results were expressed as mean ± standard deviation. The significance threshold was set at 5%, and SPSS 20.0 statistical software was used for data analysis.

## RESULTS

**Effects of aqueous and ethanolic ginger extracts on serum progesterone concentration**

**Fig.1** shows the influence of different doses of aqueous and ethanolic extracts of ginger on the serum progesterone level in female African giant rats in captivity. It is noted that except for females having received 750 mg/kg bw of aqueous extract, the serum progesterone level was significantly ( $P<0.05$ ) high in treated animals compared to the control.

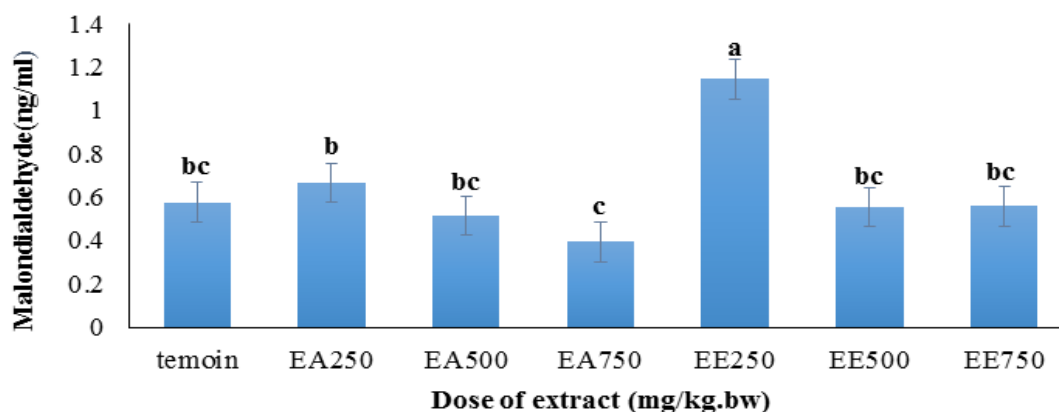


**Fig.1:** Effects of different doses of aqueous and ethanolic extracts of ginger on serum concentration of progesterone in female African giant rats in captivity.

a, b, c, d, e, f: on the diagram, the values assigned to different letters are significantly different ( $p<0.05$ ); (AE250, AE500, AE750): aqueous extract of ginger powder at respective doses of 250, 500 and 750 mg/kg.bw. ; (EE250, EE500, EE750): ethanolic extract of ginger powder at respective doses of 250, 500 and 750 mg/kg.bw.

**Influence of aqueous and ethanolic ginger extracts on oxidative stress markers concentrations**

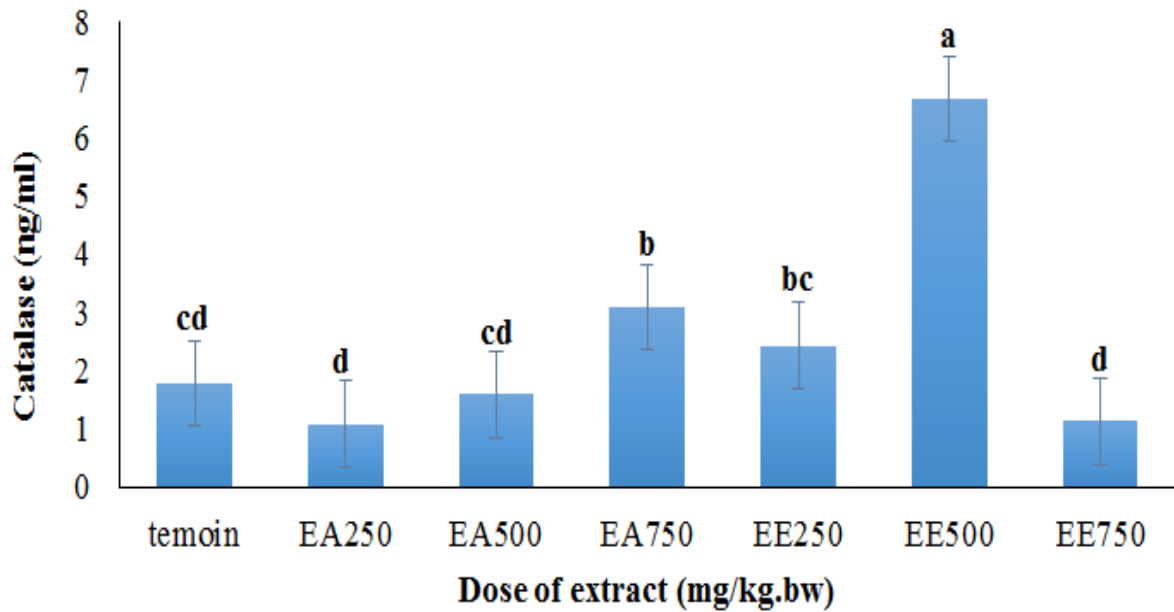
Administration of the ethanolic extract at a dose of 250 mg/kg bw induced a significant increase ( $P<0.05$ ) in malondialdehyde. The other groups obtained values comparable to that of the control (**Fig. 2**).



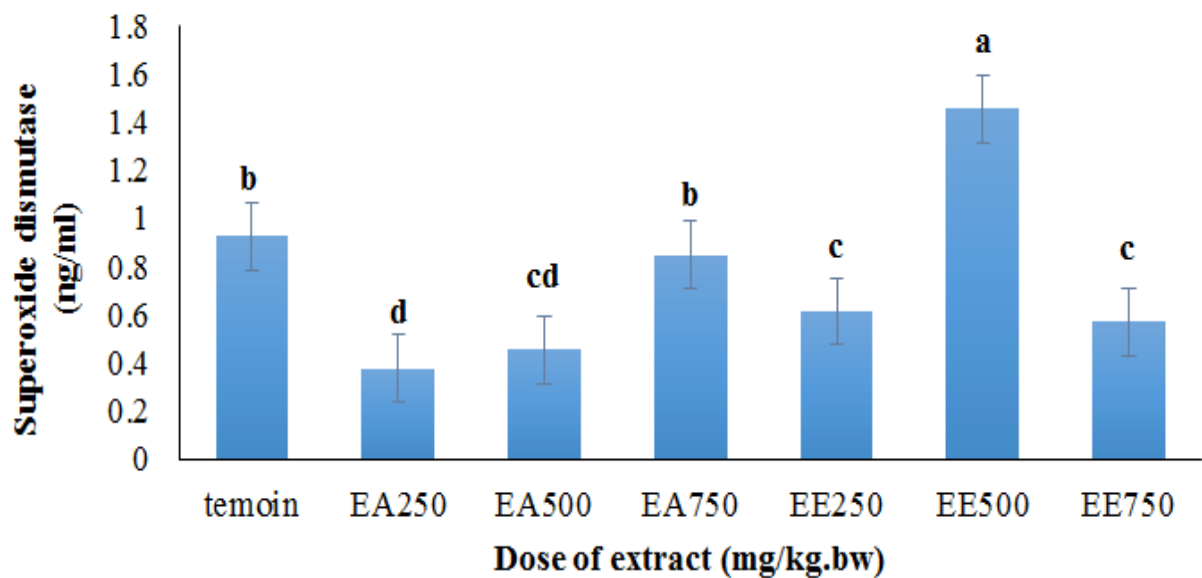
**Fig.2:** Effects of different doses of aqueous and ethanolic extracts of ginger on the level of malondialdehyde in female African giant rats in captivity.

a, b, c: on the diagram, the values assigned to different letters are significantly different ( $p<0.05$ ); (AE250, AE500, AE750): aqueous extract of ginger powder at respective doses of 250, 500 and 750 mg/kg.bw; (EE250, EE500, EE750): ethanolic extract of ginger powder at respective doses of 250, 500 and 750 mg/kg.bw.

The levels of catalase and superoxide dismutase were significantly ( $P<0.05$ ) high for females having received 750 mg/kg bw of ethanolic extract compared to females from all other groups (**Figs.3 and 4**).

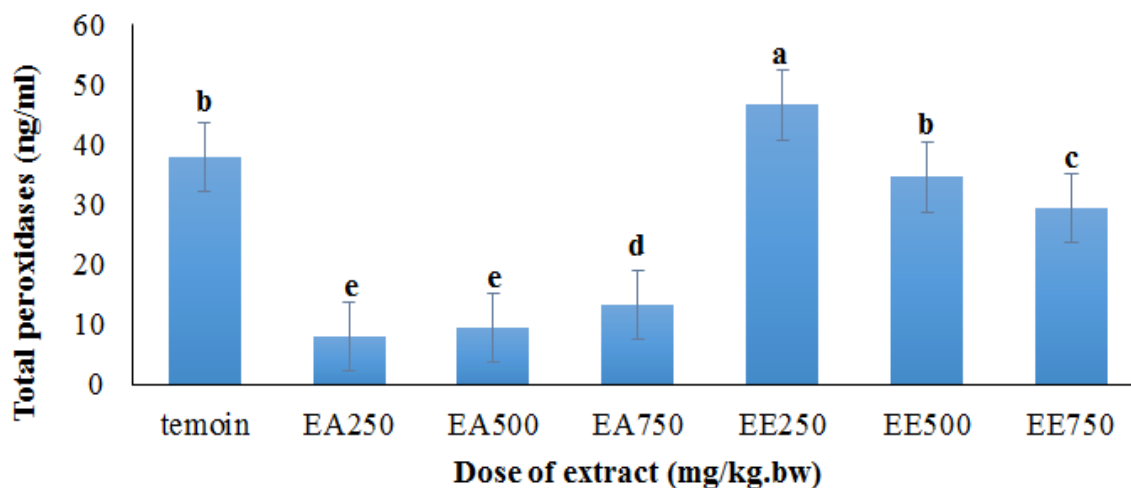


**Fig. 3:** Evolution of the catalase level following the different doses of aqueous and ethanolic extracts of ginger in female African giant rats in captivity.  
a, b, c, d: on the diagram, the values assigned to different letters are significantly different ( $p < 0.05$ ); (AE250, AE500, AE750): aqueous extract of ginger powder at respective doses of 250, 500 and 750 mg/kg.bw; (EE250, EE500, EE750): ethanolic extract of ginger powder at respective doses of 250, 500 and 750 mg/kg.bw.



**Fig.4:** Effect of aqueous and ethanolic extracts of ginger on the concentration of superoxide dismutase in female African giant rats in captivity.  
a, b, c, d: on the diagram, the values assigned to different letters are significantly different ( $p < 0.05$ ); (AE250, AE500, AE750): aqueous extract of ginger powder at respective doses of 250, 500 and 750 mg/kg.bw; (EE250, EE500, EE750): ethanolic extract of ginger powder at respective doses of 250, 500 and 750 mg/kg.bw.

The total peroxidases level was significantly ( $p < 0.05$ ) higher for females having received 250 mg/kg bw of ethanolic extract compared to females in the other groups (Fig.5).



**Fig.5:** Effects of different doses of aqueous and ethanolic extracts of ginger on the level of total peroxidases in female African giant rats.

a, b, c, d: on the diagram, the values assigned to different letters are significantly different ( $p < 0.05$ ); (AE250, AE500, AE750): aqueous extract of ginger powder at respective doses of 250, 500 and 750 mg/kg.bw; (EE250, EE500, EE750): ethanolic extract of ginger powder at respective doses of 250, 500 and 750 mg/kg.bw.

## DISCUSSION

In the present work, aqueous and ethanolic extracts of ginger increased serum progesterone levels in pregnant rats. An increase in progesterone production after fertilization is necessary for blastocyst implantation and the maintenance of gestation in mammals. During gestation, the steroidogenic function in the latter is generally carried out by the corpus luteum, then by the adrenal cortex and the fetoplacental unit (Gayrard, 2007). These results are consistent with our expectations and corroborate those of Sirotkin et al., (2018), demonstrating that the ovaries of rabbits treated *in vitro* with *Curcuma longa* powder (Zingiberaceae) release more progesterone. Indeed, the alkaloids present in ginger extracts increase the body's cholesterol level (the obligatory substrate of steroidogenesis) and consequently the progesterone level (Yakubu and Akanji, 2011).

Gestation generates free radicals (superoxides ( $O_2^-$ ) and hydrogen peroxides ( $H_2O_2$ )) in the uterus, following the implantation of the egg, the adaptation of placental and fetal circulation, the development of very dense capillary networks, and an increase in the permeability of the membranes necessary for the passage of nutrients to the embryo (Arousseau et al., 2004). These radicals are normally controlled by the body's antioxidant enzymes (catalase, superoxide dismutase, total peroxidases, etc.), which have high levels in the blood in cases of stress. Polyunsaturated fatty acids represent the main target of free radicals

through the process of lipid peroxidation (Gutteridge and Halliwell, 2000); hence, the increase in the hepatic content of malondialdehyde, which is the final product of lipid peroxidation. The results of this study show that, in general, ginger extracts had no effect on markers of oxidative stress. These results are identical to those of Dongmo et al., (2021), who used avocado pit extracts in guinea pigs. However, contradictory to the results, Tchoffo et al., (2018) and Alaeldin et al., (2021) used ginger essential oil and extract to lower malondialdehyde levels and increase superoxide dismutase, catalase and reduced glutathione levels.

This difference could be due to the harvest season of the plant material (dry season) or the different extraction methods. We found that the ethanolic extract of ginger seems to have a higher antioxidant power than that of the aqueous extract. This difference may be due to the fact that there are more active components well extracted by the ethanolic extract than in the aqueous ginger extract. What has also been proven by the phytochemical test is that the ethanolic extract of ginger contains sterols and triterpenoids, which are absent in the aqueous extract.

## CONCLUSION

Aqueous and ethanolic extracts of ginger can be used to increase in serum progesterone levels during gestation in *Cricetomys gambianus*, at doses between 250 and 500 mg/kg bw.

## Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

## REFERENCES

- ALAAELDIN, A.H., GEHAM, H.H., SALSABIL, H., ALLI, A., and AMR, A., 2021. Standardized extract of ginger ameliorates liver cancer by reducing proliferation and inducing apoptosis through inhibition oxidative stress/ inflammation pathway. *Journal of medicine and Pharmacotherapy* 134, 111102. <https://doi.org/10.1016/j.biopha.2020.111102>.
- AMIR, M.A.H., MOHAMMAD, H.A., REHAM, D.A., AYMAN.M.M., 2018. Ginger alleviates hyperglycemia-induced oxidative stress, inflammation and apoptosis and protects rats against diabetic nephropathy. *Biomedicine & Pharmacotherapy* 106, 381-389. <https://doi.org/10.1016/j.biopha.2018.06.148>.
- AUROUSSEAU, B., DURAND, D., and GRUFFAT, D., 2004. Contrôle des phénomènes oxydatifs pendant la gestation chez les monogastriques et les ruminants. *NRA Productions. Animales* 17(5), 339-354. <https://doi.org/10.20870/productions-animales.2004.17.5.3607>.
- BAKKALI, F., AVERBECK, S., AVERBECK, D., and IDAOMAR, M., 2008. Biological effects of essential oils. *Food and Chemical Toxicology* 46, 446-475. <https://doi.org/10.1016/j.fct.2007.09.106>.
- BUSMAN, H., KANEDI, M., 2016. Testicular Function of Rats Treated with Water Extract of Red Ginger (*Zingiber officinale var rubrum*) Combined with Zinc. *Journal of Food and Nutrition Research* 4(3), 157-162. <https://doi.org/10.12691/jfnr-4-3-5>.
- DONGMO, N.A.B., VEMO, B.N., TCHOFFO, H., LONTIO, F.A., MENKEM, B., DJUISSI, M.N., MOHAMADOU, A., MAHAMAT, T.M.A., CHONGSI, M.M.M., and NGOULA, F., 2021. Oxidative status and reproductive characteristics in female cavies (*Cavia porcellus* L.) Fed on aqueous extract of avocado (*persea americana* mill.) Seed. *Journal of Veterinary Healthcare* 2(3), 17-28. <https://doi.org/10.14302/issn.2575-1212.jvhc-21-3759>.
- FLAVIEN, B., 2023. Physiologie de la grossesse Pregnancy physiology. *Actualités Pharmaceutiques* 62(628), 18-21. <https://doi.org/10.1016/j.actpha.2023.06.005>.
- FONKEM, S., TSAMBOU, M.A.M., FONO, T.L., FODA, F.C., VEMO, B.N., TAKAM, M.B.I., KOUAMO, J., and KENFACK, A., 2022. 17- $\alpha$ -hydroxyprogesterone caproate improves female African giant rats (*Cricetomys gambianus*) fertility in captivity. *Journal of Agriculture and Veterinary Science* 15(3), 15-21. <https://doi.org/10.9790/2380-1503031521>.
- GHOLAMI-AHANGARAN, M., KARIMI-DEHKORDI, M., JAVAR, A.A., SALEHI, M.H., and OSTADPOOR, M., 2021. A systematic review on the effect of Ginger (*Zingiber officinale*) on improvement of biological and fertility indices of sperm in laboratory animals, poultry and humans. *Veterinary Medicine and Science* 7(5), 1959-1969. <https://doi.org/10.1002/vms3.538>.
- GUTTERIDGE, J., and HALLIWELL, B., 2000. Free radicals and antioxidants in the year 2000: a historical look to the future. *Annals of the New York Academy of Sciences* 899(1), 136-147. <https://doi.org/10.1111/j.1749-6632.2000.tb06182.x>.
- HABBU, P.V., SHASTRY, R.A., MAHADEVAN, K.M., HANUMANTHACHAR, J., and DAS, S.K., 2008. Hepatoprotective and antioxidant effects of *Argyrea speciosa* in rats. *African Journal of Traditional Complementary and Alternative Medicine* 5(2), 158-164. <https://doi.org/10.4314/ajtcam.v5i2.31268>.
- KAÏS, H.A-G., PAUL, A.F., and CATHERINE, G., 2010. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *International Journal of Biochemistry and Cell Biology* 42(10), 1634-1650. <https://doi.org/10.1016/j.biocel.2010.06.001>.
- MISRA, H.P., and FRIDOVICH, I., 1972. The generation of superoxide radical during the autoxidation of hemoglobin. *Journal of Biological Chemistry* 247(21), 6960-6962. [https://doi.org/10.1016/S0021-9258\(19\)44679-6](https://doi.org/10.1016/S0021-9258(19)44679-6).
- MUSA, Y., MUSBAU, A.A., TEMIDAYO, O., 2006. Aphrodisiac potential of the extract of *Fadogia agrestis* (Scweinf Ex Hiern) stem in male albino rats. *ResearchGate* 7(4), 399-404. <https://doi.org/10.1111/j.1745-7262.2005.00052.x>.
- MUTINATI, M., PICCINNO, M., RONCETTI, M., CAMPANILE, D., RIZZO, A., and SCIORSCI, R.L., 2013. Oxidative stress during pregnancy in the sheep. <https://doi.org/10.1111/rda.12141>.
- NAFIYE, Y., 2018. Ginger (*Zingiber officinale*) might improve female fertility: A rat model. *Journal of the Chinese Medical Association* 81, 905-911. <https://doi.org/10.1016/j.jcma.2017.12.009>.
- NILSSON, U.A., OLSSON, L.I., CARLIN, G., and BYLUND-FELLENUS, A.C., 1989. Inhibition of lipid peroxidation by spin labels, *Journal of Biological Chemistry* 264(19), 11131-11135. [https://doi.org/10.1016/S0021-9258\(18\)60439-9](https://doi.org/10.1016/S0021-9258(18)60439-9).
- SHANMUGAM, M., KORUVI, M., NISHANTH, K. and KERSIREDDY, S.R., 2011. Neuroprotective effect of ginger on anti-oxidant enzymes in streptozotocin-induced diabetic rats. *Food and Chemical Toxicology* 49(4), 893-897. <https://doi.org/10.1016/j.fct.2010.12.013>.
- SINHA, A.K., 1972. Colorimetric assay of catalase. *Analytical Biochemistry* 47(2), 389-394. [https://doi.org/10.1016/0003-2697\(72\)90132-7](https://doi.org/10.1016/0003-2697(72)90132-7).
- SIROTKIN, A.V., KODAS, A., STOCHR, A., BALAZI, A., FOLDES, M., MAKOV, P., CHRENE, P., and HARRATH, A.H., 2018. Effect of turmeric on the viability, ovarian folliculogenesis, ovarian hormones and reponse to luteinizing of rabbits. *Animal* 12, 1242-1249. <https://doi.org/10.1017/S175173111700235X>.
- STENQVIST, A., OLESZCZUK, K., LEIJONHUFVUD, I.I., GIWERCMAN, A., 2018. Impact of antioxidant treatment on DNA fragmentation index: a double-blind placebo-controlled randomized trial. *Andrology* 6(6), 811-816. <https://doi.org/10.1111/andr.12547>.
- STEPHANIA, T., 2015. Physiology, production and action of progesterone. *Reproductive Medicine Unit, GynePro Medical Group, Bologna, Italy* 94, 8-16. <https://doi.org/10.1111/aogs.12771>.
- TAPSELL, L.C., HEMPHILL, I., COBIAC, L., PATCH, C.S., SULLIVAN, D.R., FENECH, M., and INGE,

- K.E., 2006.** Health benefits of herbs and spices: The past, the present, the future. Australian Medical Research 185, 4-24. <https://doi.org/10.5694/j.1326-5377.2006.tb00548>.
- TCHOFFO, H., KANA, J.R., NGOULA, F., FOLACK, T.L.V., ADOUM, G., MOUSSA, M.O., and NGOUOZEU, M.W.M., 2018.** Growth performance, serum biochemical profile, oxidative status and fertility traits in male japanese quail fed on ginger (*Zingiber officinale roscoe*) essential oil. University of Dschang. Veterinary Medicine International. 7682060. <https://doi.org/10.1155/2018/7682060>.
- YAKUBU, M.T., and AKANJI, M.A., 2011.** Effect of aqueous extract of *Massularia acuminata* stem on sexual behaviour of male Wistar rats. Evidence-Based Complementary and Alternative Medicine. 10 pp. <https://doi.org/10.1155/2011/738103>.
- ZHAO, X., YANG Z.B., YANG, W.R., WANG, Y., JIANG, S.Z., and ZHANG, G.G., 2011.** Effects of ginger roots (*Zingiber officinale*) on laying performance and antioxidant status of laying hens and on dietary oxidation stability. Poultry Science 90, 1720-1727. <https://doi.org/10.3382/ps.2010-01280>.