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Oxidative Status and Reproductive Characteristics in Female Cavies (*Cavia porcellus* L.) Fed on Aqueous Extract of Avocado (*Persea americana* Mill.) Seed

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Abstract

The present study was initiated to improve the farm animals' productivity through the use of medicinal plants. More specifically, to determine in female cavies the effects of aqueous extract of avocado seed powder (AEASP) on the estrous cycle, the levels of LH, estradiol and tissues (ovarian and uterine) biomarkers of oxidative stress. For the trial, 24 female cavies with regular estrous cycles were selected among 40 trough observation of 4 estrous cycles. They were randomly shared into 4 groups of 6 females each, comparable in term of body weight (bw) (463.60±77.69 g). They received by gavage 1 mL/kg bw of distilled water for the control and 100, 200, 400 mg/kg bw of AEASP respectively for the groups EA100, EA200 and EA400. Subsequently, 3 estrous cycles were studied every day during all the treatment period. At the end, the cavies were slaughtered at the estrus phase; blood, ovaries and uterus were collected for analysis. As result, the AEASP significantly ($p<0.05$) increase the duration of the estrus phase in females of group EA100, without affecting significantly the duration of the estrous cycle as referred to the control. It significantly reduce the serum level of total cholesterol and increase ($p<0.05$) the serum concentration of LH in cavies of group EA100 compared to the control. AEASP significantly increase the serum concentration of estradiol in all treated females as referred to the control. It significantly increase the level of malondialdehyde (MDA) in the ovaries of the females of group EA400. In the uterine tissue, superoxide dismutase (SOD) increase significantly in the cavies of group EA200 compare to the control. We can conclude that the AEASP increase the duration of the estrus phase of cavies without affecting the duration of the estrous cycle. Subsequently, it increases the serum concentration of LH and estradiol.

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Introduction

With a view of promoting health and well-being of farm animals and thus increase their reproduction performances in developing countries such as Cameroon [1], farmers used drugs based on synthetic molecules such as antibiotics and hormones, In face of their adverse effects on health and well-being of animals as well as animal products consumers, the new tendency is the replacement of those synthetic molecules by bioactive molecules of natural origin, derivate from plants and agro-food industries wastes [2]. They are known to be available, less dangerous, less expensive and capable of inducing pharmacological effects similar to those of conventional medicines based on synthetic molecules. It is the case of avocado seeds.

Persea americana Mill., commonly known as avocado or pear is a plant originated from Mexico, belonging to the *Lauraceae* family. Botanically, his fruit is a berry with a single large seed [3]. Usually considered as "waste" it contained a large quantity of bioactive phytochemicals such as polyphenols, phytosterols, triterpenoids, flavonoids, carotenoids, alkaloids, saponins, tannins, proteins, fatty acids, vitamins (A, C and E) and many others [4, 5, 6]. Those bioactive compounds conferred to avocado seeds many pharmacological properties. [7] demonstrated the hypocholesterolenic and antioxidant effects induced by Beta sitosterol and tocopherol present in aqueous extract of avocado seeds in rats. Also, [8] proved the ability of some bioactive compounds present in avocado seed to protect spermatozoids contained in goat semen against oxidative stress during it cryopreservation [9] demonstrated that aqueous and ethanolic extracts of *Persea americana* seed exhibited antimicrobial activities against pathogens such as; *Escherichia coli* and *Staphylococcus aureus.*

Very few studies to our knowledge focus on the effects of avocado seeds on the reproduction of females mammals; it is why this study was initiated with the aim of contributing to the improvement of the productivity of farm animals through the use of medicinal plants. More specifically, to determine in female cavies (*Cavia porcellus* L.) the effects of ethanolic extract of avocado seed powder on the estrous cycle, the levels of some hormones linked to reproduction (cholesterol, LH and estradiol) and tissues (ovarian and uterine) biomarkers of oxidative stress.

Materials and Methods

Animal Material

Twenty-four (24) adult female's cavies raised in the teaching and research farm of the University of Dschang were used. They were comparable in terms of body weight (463.60±77.69 g) and maintained during the test under the conditions of experimentation and handling in accordance with internationally accepted standard ethical guidelines for laboratory animal used and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.

Plant Material

Avocados were harvested on the same tree in Mbouda (Bamboutos division in the West region of Cameroon) and authenticated in the National Herbarium of Yaounde (Cameroon) under the number 18604/SRF/CAM, in referred to Daniel Dong material number 80. The seeds were extracted from the fruits, cleaned and grown in fine particles, then directly boiled at 100°C for 15 minutes (in a constant pressure pot) and dried at 60° C (under shade) in reference to [10] method, those to reduce the levels of anti-nutritional factors. The dried seeds were crushed to obtain homogeneous powder

used for the preparation of the aqueous extract.

Preparation of the Extract

The aqueous extract of avocado seed powder was realised according to the maceration method described by [11]. For this purpose, 500 g of avocado seed powder were introduced into 2000 ml of distilled water and macerated during 24 hours and thereafter, the homogenate was filtered with No 1 Whatmann filter paper. The filtrate obtained was dried at 45°C in the oven, packed into dark sample bottles and stored at 4°C in a refrigerator until used. Subsequently, a phytochemical screening were realised to determine the chemical composition of the extract (Table 1).

Housing and Feeding

During the test period, twenty-four (24) female's cavies with normal (regular) estrous cycles were divided into 4 groups of 6 females each, kept in 4 cages (6 females/cage). Each of these cages, measuring 1 m in length, 60 cm in width and 60 cm in height and lined with a layer of untreated white wood cheeps 5 cm thick. These cages were each equipped with a wooden feeder (60 cm long, 10 cm wide and 5 cm deep) and a plastic drinker (50 cl). Animals were introduced two weeks after disinfection. Throughout the trial period, all the cavies were given free access and *ad libitum* to potable water and a diet containing 97.82% dry matter, 86.06% organic matter, 16.79% crude protein, 15.80% crude fiber and 13.94% ash.

Experimental Protocol

After 2 weeks of adaptation, twenty-four (24) cavies with normal estrous cycles were selected among 40 females after observation of four consecutive estrous cycles (17 days x 4 = 68 days approximately). They were randomly allocated to 4 groups of 6 females each, comparable in terms of body weight corresponding to the different treatments. The control group, receiving 1 mL of distilled water and groups EA100, EA200 and EA400 receiving respectively 100, 200 and 400 mg/kg bw of aqueous extract of avocado seed powder. The treatment (oral administration) was done every morning (between 6 a.m. and 8 a.m.) and subsequently vaginal smears were carried out on the same subjects (between 8 a.m. and 9 a.m.) during 3 estrous cycles (about 51 days).

Performing Vaginal Smears

The vaginal fluid of each cavy was taken every morning between 8 a.m. and 9 a.m. using a pipette containing 0.25 mL of NaCl (0.9%), then spread on a slide and left a few with sample was introduced in methanol 70% (5 minutes) for fixation and stained with methylene blue 0.5% (10 minutes) to colour the cells. The excess was removed by rinsing with tap water. The proportion of three different types of cells present on the slide (round nucleated epithelial cells, non-nucleated cornified epithelial cells and leucocytes), observed under microscope (10 and 40 X objective lenses) was used for the determination of the estrous cycle phases as illustrated on figure 1 [12] and. figure 2.

Organs Collection and assessment of Biochemical Parameters

At the end of the treatment, all cavies of each group (6/batch) were sacrified at the estrous phase. Blood samples were collected after sectioning the jugular vein, in dry tubes, centrifuged at 3000 rpm for 5 minutes and the collected serum was stored at -20°C until use. Part of this serum was used for the determination of total cholesterol, in accordance with the colorimetric method described in the leaflet of CHRONOLAB kit and the other part for the assay of reproductive hormones, estradiol and LH, in accordance with the ELISA (enzyme-linked immunosorbent assay) methods described in the leaflets of the DRG and Omega DIAGNOSTICS kits. The ovaries and uteri were collected after laparotomy, weighed. For each female, the left ovary and the left uterine horn were ground separately in porcelain mortars, so as to obtain the 15% homogenates, using Tris buffer (pH 7.4). The ground material was then centrifuged at 3000 rpm for 30 min using a cold centrifuge brand. The supernatant collected was distributed in labelled Eppendorf microtubes and stored at -20°C and subsequently used for the determination of oxidative stress indicators, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and total peroxidases (POX) according to the methods described respectively by [13]; [14]; [15] and [16]; And for the determination of total tissue proteins in accordance with the colorimetric method described in the leaflet of CHRONOLAB kit.

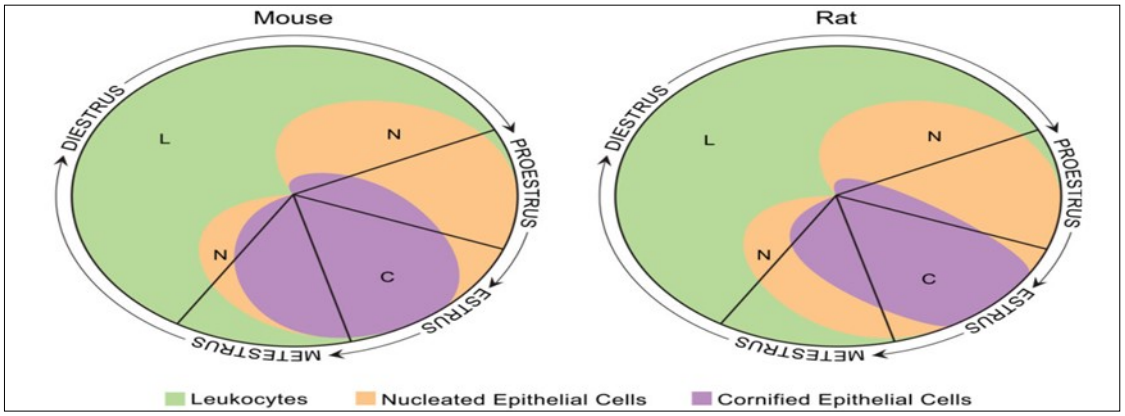

Figure 1. Relative proportion of the different types of cells present in a vaginal smear of rat and mouse [12].

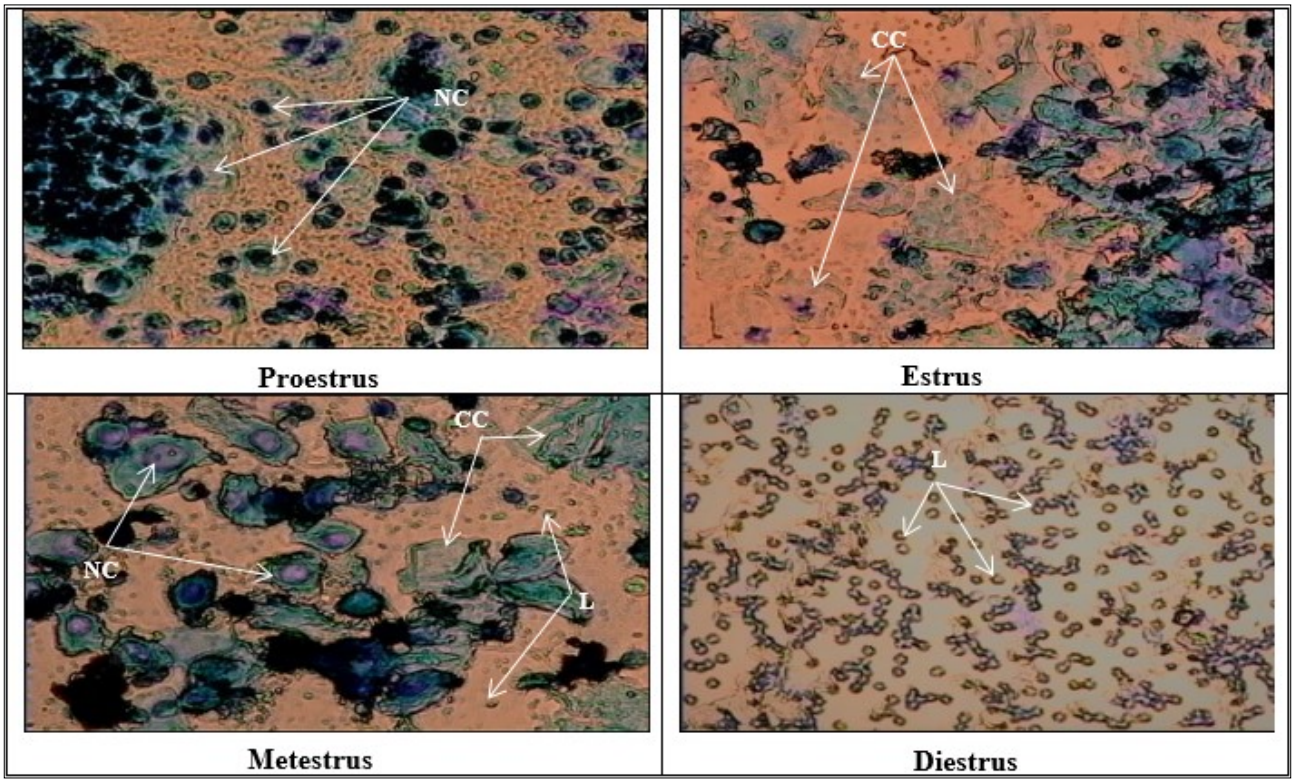

Figure 2. Photomicrographs of vaginal smear from cavies at proestrus, estrus , metestrus and diestrus phases observed under a light microscope at 40 x objective lens.

NC= Round nucleated epithelial cells, CC= Cornified non nucleated epithelial cells L= Leucocytes.

Table 1. Phytochemical constituents of aqueous extract of avocado seed powder

Constituent	Aqueous extract	Test realised
Flavonoids	+	Shinada test
Alkaloids	-	Dragendorff test
Phenols	++	Ferrical Chloride test
Triterpens	+	Lieberman Buchard test
Steroids	+	Lieberman Buchard test
Saponins	-	Foam test
Tannins	++	Ferrical Chloride test

- : absent; +: present; ++: present in high quantity

Statistical Analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) to test the effect of different doses of aqueous extract of avocado seed powder on the studied parameters. Duncan's test was used to separate the means when there were significant differences. The results were expressed as mean±standard deviation and the significance level fixed at 5%. SPSS 25.0 statistical software was used for data analysis.

Results and Discussion

Results

Effects of Aqueous Extract of Avocado Seed Powder on the mean Duration of the Different Phases of Estrous Cycle in Cavies

From Figure 3, it emerges that aqueous extract of avocado seed powder treatment induced a significant ($p<0.05$) increase of the mean duration of the estrus phase only in the group receiving 100 mg/kg. bw compared to the control. However, the proestrus, metestrus and diestrus was not significantly ($p>0.05$) affected by this treatment in reference to the control.

Effects of Aqueous Extract of Avocado Seed Powder on the Duration of the Estrous Cycle in Cavies

The effects of aqueous extract of avocado seed powder on the duration of the estrous cycle in cavies is presented in Table 2. It appear that the oral administration of aqueous extract of avocado seed powder did not induced a significant ($p>0.05$) effects on the duration of the 1st, 2nd and 3rd cycles in reference to the control.

Effects of Aqueous Extract of Avocado Seed Powder on Relative Weights of Organs (Ovaries and Uterus) and Uterus Measurements in Cavies.

Table 3 shows the effects of aqueous extract of avocado seed powder on relative weights of organs (ovaries and uterus) and uterus measurements. It appears that the treatment did not significantly ($p>0.05$) affect the relative weights, length, width and thickness of the uterus in reference to the control.

Effects of Aqueous Extract of Avocado Seed Powder on Serum Levels of total Cholesterol and reproductive hormones (Estradiol and LH) in cavies.

From table 4, it appeared that the treatment induced a general decrease of the total cholesterol level in all the treated groups. However, this decrease was only significant ($p<0.05$) in the females receiving 100 mg/kg bw of the extract compared to the control. LH and estradiol levels increase in all the groups receiving aqueous extract of avocado seed powder. This increase in LH level was only significant ($p<0.05$) in female cavies treated with the dose 100 mg/kg bw compared to the control; while estradiol level significantly ($p<0.05$) increase in all the treated groups compared to the control.

Effects of Aqueous Extract of Avocado Seed Powder on Tissues Biomarkers of Oxidative Stress in Female Cavies

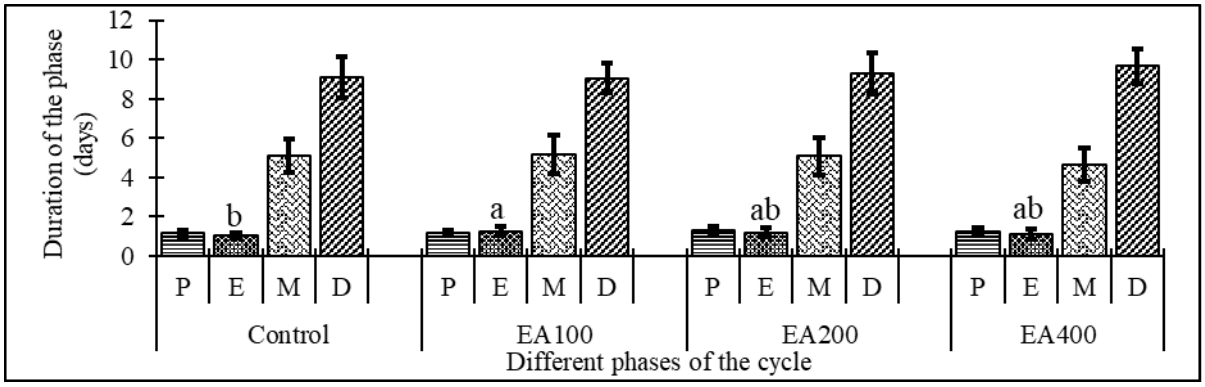

Figure 3. Effects of aqueous extract of avocado seed powder on the mean duration of the different phases of estrous cycle in cavies.

(^{a,b}): on the same histogram, the bars with different letters are significantly different ($p<0.05$) ; Control : 1 mL/kg pc of distilled water ; (EA100, EA200, EA400) :aqueous extract of avocado seed powder at the respective doses of 100, 200 and 400 mg/kg bw.

Table 2. Effects of aqueous extract of avocado seed powder on the duration of 3 consecutive estrous cycles in cavies.

Duration of the estrous cycles (days)	Control (n=6)	Doses of aqueous extract of avocado seed powder (mg/kg bw)			p-value
		EA100 (n=6)	EA200 (n=6)	EA400 (n=6)	
1st cycle	16.89±1.22	16.50±0.97	16.94±1.67	16.89±0.96	0.86
2nd cycle	16.11±0.82	17.05±1.65	16.72±1.32	16.39±1.05	0.42
3rd cycle 3	16.22±1.09	16.33±1.09	16.89±0.89	16.67±0.61	0.43
Average of the 3 cycles	16.41±0.86	16.63±1.05	16.85±0.88	16.65±0.68	0.76

n= number of samples, (^{a,b}) : on the same line, the values assigned to different letters are significantly different ($p<0.05$) ; p= probability ; Control : 1 mL/kg bw of distilled water ; (EA100, EA200, EA400) :aqueous extract of avocado seed powder at the respective doses of 100, 200 and 400 mg/kg bw.

Table 3. Effects of aqueous extract of avocado seed powder on relative weights of organs (ovaries and uterus) and uterus measurements in cavies.

Relative weights of organs (mg/kg bw) and uterus measurements (Cm)	Control (n=6)	Doses of aqueous extract of avocado seed powder (mg/kg bw)			p-value
		EA100 (n=6)	EA200 (n=6)	EA400 (n=6)	
Relative weights of organs					
Ovaries	10.89±2.53	10.43±3.02	10.30±1.91	08.72±1.46	0,41
Uterus	213.13±33.40	201.77±32.08	232.16±49.74	248.81±30.40	0.17
Uterus measurements					
Length	4.72±0.73	5.02±0.78	4.55±0.55	5.03±0.61	0.54
Width	0.75±0.05	0.71±0.05	0.68±0.07	0.77±0.08	0.15
Thickness	0.63±0.07	0.55±0.06	0.60±0.06	0.57±0.08	0.24

n= number of samples, (^{a,b}) : on the same line, the values assigned to different letters are significantly different ($p<0.05$) ; p= probability ; Control : 1 mL/kg bw of distilled water ; (EA100, EA200, EA400) :aqueous extract of avocado seed powder at the respective doses of 100, 200 and 400 mg/kg bw.

Table 4. Effects of aqueous extract of avocado seed powder on serum levels of Total cholesterol and reproductive hormones (estradiol and LH) in cavies.

Biochemical parametres	Control (n=6)	Doses of aqueous extract of avocado seed powder (mg/kg bw)			p-value
		EA100 (n=6)	EA200 (n=6)	EA400 (n=6)	
Total cholesterol (mg/dL)	62.19±6.53^{a}	44.09±10.82^{b}	59.91±8.00^{a}	60.04±3.05^{a}	0.00
LH (mUI/mL)	5.63±1.29^{b}	10.40±2.70^{a}	6.80±1.30^{b}	7.67±0.58^{b}	0.00
Estradiol (pg/mL)	80.00±14.14^{c}	115.00±9.85^{a}	98.20±8.87^{b}	99.50±9.88^{b}	0.00

n= number of samples, (^{a,b,c}) : on the same line, the values assigned to different letters are significantly different ($p<0.05$) ; p= probability ; Control : 1 mL/kg bw of distilled water ; (EA100, EA200, EA400) :aqueous extract of avocado seed powder at the respective doses of 100, 200 and 400 mg/kg bw.

Case of Ovarian Tissue

The effects of aqueous extract of avocado seed powder on biomarkers of oxidative stress in the ovarian tissue of cavies is shown in Table 5. It appears that in exception of the dose 200 mg/kg bw of aqueous extract of avocado seed powder that induced a significant ($p<0.05$) increase of ovarian total cholesterol level in reference to the control in cavies, the other doses did not have significant ($p>0.05$) effect on it. The treatment induced a general increase in MDA level but this increase was only significant ($p<0.05$) in females receiving the highest dose (400 mg/kg bw) in reference to the control. Catalase, SOD and POX activities were not significantly ($p>0.05$) affected by the oral administration of aqueous extract of avocado seed powder in cavies compared to the control.

Case of Uterine Tissue

The effects of aqueous extract of avocado seed powder on biomarkers of oxidative stress in the uterine tissue of cavies is summarised in Table 6. It appears that the SOD level increases in all the treated groups. This increase was only significant ($p<0.05$) in the group EA200 compared to the control. The other considered parameters were not significantly ($p>0.05$) affected by the different doses of aqueous extract of avocado seed powder.

Effects of Aqueous Extract of Avocado Seed Powder on Serum Proteins Levels in Cavies

From Table 7, the treatment whatever the dose did not induced a significant ($p>0.05$) effect on serum total protein, albumin and globulin levels.

Discussion

Phytochemical screening of aqueous extract of avocado seed powder carried out in this study revealed the presence of flavonoids, phenols, triterpenes, steroids and tannins. These bioactive molecules are reputed to have diverse pharmacological properties such as steroidogenic, antioxidant, antibiotic, immunomodulatory and so on [17]. Based on these activities, the present study was initiated to evaluate the effects of aqueous extract of avocado seed powder on the estrous cycle, the levels of some reproductive hormones (LH and estradiol) and tissues (ovarian and uterine) biomarkers of oxidative stress.

The estrous cycle consists of a definite sequence of events that affect the anatomical, physiological and behavioural of reproductive organs. It is divided into four phases named: proestrus, estrus, metestrus and diestrus. Ovulation usually occurs between the beginning of proestrus and the end of estrus [18]. It appears in the current study that the oral administration of aqueous extract of avocado seed powder increased the duration of the estrus phase in the cavies of all the treated groups. This result is in accordance with the one obtained by [19] on female rats with normal estrous cyclicity after administrating the aqueous extract of *Ficus asperifolia* at the dose 100 mg/kg bw and contradict the one obtained by [20] after administrating 400 mg/kg bw of aqueous extract of *Cynodon dactylon* to females Wistar rats. Phytoestrogens are molecules present in plants that promoted estrogenic actions in animals. Because of their structure similar to those of estrogens, they are capable to bind one estrogens receptors and act as estrogens agonist or antagonist depending to their concentration in the blood [21]. In this study, some phytoestogens present in aqueous extract of avocado seed acted as estrogen agonist and could have induced estrus in cavies [22]. The sexual activity in mammals, including heat is under the control of gonadic hormones (estrogens and progesterone), and gonadotropins hormones such as LH and FSH. In the current study, avocado seed extract increase the levels of estradiol and LH in the cavies at the dose 100 mg/kg bw. Similar result was obtained by [23] with the administration of aqueous extract of *Myrianthus arboreus* leaves at the dose 200 mg/kg bw in female Wistar rats. It also corroborated the one obtained by [24] after administrating methanolic extract of *Andrographis paniculata* to female albino rats at the dose 500 mg/kg bw. In fact, phytoestrogens present in plants have the ability to stimulate or inhibit by feedback reaction of LH, FSH and estrogens. In the case of this study, those molecules present in avocado seed extract could have induce by positive feedback reaction of LH, hence the increase in its rate. The LH subsequencely stimulates the production of estradiol [25]. Cholesterol is the precursor of steroid hormones, including estrogens [26]. Aqueous extract of avocado seed powder induce the reduction of total cholesterol level at the dose 100 mg/kg bw in cavies. This recorded result is in accordance with the

Table 5. Effects of aqueous extract of avocado seed powder on biomarkers of oxidative stress in the ovarian tissue of cavies.

Oxidative stress markers	Control (n=6)	Doses of aqueous extract of avocado seed powder (mg/kg bw)			p-value
		EA100 (n=6)	EA200 (n=6)	EA400 (n=6)	
Total protein (g/dL)	1.36±0.09^{bc}	1.68±0.33^{ab}	2.01±0.35^{a}	1.33±0.03^{c}	0.00
MDA (µM)	0.53±0.06^{b}	0.53±0.11^{b}	0.70±0.21^{ab}	0.87±0.20^{a}	0.00
SOD (U/min/g of ovarian proteins)	0.83±0.23	0.80±0.08	0.68±0.05	0.83±0.98	0.31
CAT (µM/min/g of ovarian proteins)	1.52±0.93	2.36±0.88	1.62±0.90	1.71±0.68	0.41
POX (mM/min/g of ovarian proteins)	6.17±2.80	8.03±2.90	6.40±2.02	8.97±1.94	0.20

Table 6. Effects of aqueous extract of avocado seed powder on biomarkers of oxidative stress in the uterine tissue of cavies.

Oxidative stress markers	Control (n=6)	Doses of aqueous extract of avocado seed powder (mg/kg bw)			p-value
		EA100 (n=6)	EA200 (n=6)	EA400 (n=6)	
Total protein (g/dL)	1.74±0.18	1.66±0.10	1.65±0.19	1.67±0.09	0.69
MDA (µM)	1.27±0.59	1.41±0.48	1.13±0.19	1.32±0.23	0.68
SOD (U/min/g of uterine proteins)	0.77±0.09^{b}	0.82±0.06^{b}	0.95±0.08^{a}	0.82±0.06^{b}	0.00
CAT (µM/min/g of uterine proteins)	1.23±0.21	1.35±0.12	1.68±0.65	1.17±0.06	0.14
POX (mM/min/g of uterine proteins)	24.89±9.69	27.82±4.35	27.79±7.77	28.25±4.45	0.83

n= number of samples, (^{a,b,c}) : on the same line, the values assigned to different letters are significantly different ($p<0.05$) ; p= probability ; Control : 1 mL/kg bw of distilled water ; (EA100, EA200, EA400) :aqueous extract of avocado seed powder at the respective doses of 100, 200 and 400 mg/kg bw. MDA:Malondialdehyde; SOD: Superoxide Dismutase; CAT: Catalase; POX: Total Peroxidase.

Table 7. Effects of aqueous extract of avocado seed powder on serum proteins level in cavies.

Proteins concentrations (g/dL)	Control (n=6)	Doses of aqueous extract of avocado seed powder (mg/kg bw)			p-value
		EA100 (n=6)	EA200 (n=6)	EA400 (n=6)	
Total protein	4.40±0.33	4.08±0.44	4.43±0.81	4.72±0.62	0.34
Albumin	2.49±0.50	2.36±0.57	2.69±0.38	2.74±0.37	0.52
Globulin	1.91±0.34	1.72±0.44	1.74±0.54	1.98±0.50	0.74

n= number of samples, (^{a,b}) : on the same line, the values assigned to different letters are significantly different ($p<0.05$) ; p= probability ; Control : 1 mL/kg bw of distilled water ; (EA100, EA200, EA400) :aqueous extract of avocado seed powder at the respective doses of 100, 200 and 400 mg/kg bw.

one obtained by [27] after administrating the aqueous extract of *Persea americana* seed extract at the doses 100 and 200 mg/kg bw to rabbit. Indeed cholesterol present in the cavies' blood could have been used during ovarian steroidogenesis as a raw material for steroids hormones synthesis. That can explain why total cholesterol level decrease while estradiol level increase in the serum of treated cavies [26].

Several studies have proved that during reproductive period (estrus, gestation, birthing and lactation), female mammals are vulnerable. This vulnerability is the result of oxidative stress. In living organisms in health, there is a state of equilibrium between reactive oxygen species and the antioxidant defences. Indeed, when it switched on the side of free radicals, it creates a situation of oxidative stress, a state of imbalance between the production of antioxidants and reactive oxygen species (ROS) in favour of the latter [28]. Free radicals are capable to denature the DNA of cells, leading to genetic mutations. The can also cause lipid peroxidation and then destroy the cell membrane. These free radicals can be teratogen and led to the deaths of the embryo when the affect germinal cells [29]. In the present study, aqueous extract of avocado seed powder increase the level of MDA in the ovarian tissue of cavies at the highest dose (400 mg/kg bw). These results are in accordance with those of [30] in the testis of male cavies after intoxicating them with 80 mg/kg bw of acetamiprid and the result obtained by [31] in rabbits ovaries after intoxicating them with 40 mg/kg bw of potassium dichromate. On the contrary, some investigators like [32] registered the reduction in the level of MDA in albino rats and cavies belonging to both sexes, after giving them by oral administration 100 and 200 mg/kg bw of hydroethanolic extract of *Ocimum sanctum* leaves. This result can be due to the negative effect of natural toxicants present in avocado seeds. In fact avocado seeds contain antinutritional compounds such as tannins, saponin and phytic acid that could have intoxicated the cavies [10]. [33] obtained 20% mortality in male mice 24 hours after giving them per gavage 500 mg/kg bw of ethanolic extract of avocado seed during an acute toxicity test performed one this plant. In the uterine tissue, aqueous extract of avocado seed powder induced an increase of SOD level in cavies at the dose 200 mg/kg bw. This result is similar to the one obtained by [32] after administrating 100 and 200 mg/kg bw of hydroethanolic extract of *Ocimum sanctum* leaves extract to albino rats and cavies, belonging to both sex. In fact avocado seeds contained some molecules as Vitamin C, E, flavonoids and phenols that possess antioxidant capacity [34]. This property could have stimulated the antioxidant defence of the cavies resulting to the increase of SOD activity [35].

Conclusions

At the end of this study, the following conclusions were made. The aqueous extract of avocado seed powder increases the duration of heat (estrous phase). It also reduced the level of total cholesterol and

increases the levels of reproductive hormones (LH and estradiol) in cavies. Aqueous extract of avocado seed powder has minors' effects on the oxidative status of the ovaries and uterine tissues.

References

1. Gourmelen C., Royer É., Salaün, Y., (2001). Impact d'une restriction de l'usage des facteurs de croissance antibiotiques sur le coût de production du porc. Institut technique du porc. Journées recherche porcine en France, 33 : 291-298.
2. Rodríguez-Carpena J.G., Morcuende D., Andrade M.-J., Kylli P., Estévez M., (2011). "Avocado (*Persea americana* Mill.) phenolics, *in vitro* antioxidant and antimicrobial activities and inhibition of lipid and protein oxidation in porcinepatties,". *J. Agr. Food Chem.*, 59(10) : 5625-5635.
3. Cowan A.K. and Wolstenholme B.N., (2016). Avocado. In encyclopedia of food and health; Caballero, B.; Finglas, P.M.; Toldrá, F. Eds.; *Academic Press: Oxford.*, UK, 294-300.
4. Flores M., Ortiz-Viedma J., Curaqueo A., Rodriguez A., Dovale-Rosabal G., Magaña F., Vega C., Toro M., López L., Ferreyra R., Defilippi B.G., (2019). Preliminary studies of chemical and physical properties of two varieties of avocado seeds grown in chile. *J. Food Qual.*, Vol. 2019, Article ID 3563750, pp: 11.
5. Deep J.B., Muhammad A., Alsherbiny A., Perera S., Low M., Basu A., Devi O.A., Barooah M.S., Li C.G., Papoutsis K., (2019). The odyssey of bioactive compounds in avocado (*Persea americana*) and their health benefits. *Antioxidants*, 8: Pp.426.
6. Uzor B.C., Nwagbo N.T., Manu A., (2016). Phytochemical composition and antimicrobial activity of *Persea americana* (avocado) seed extract against selected clinical isolates. *Nig. J. Microbiol.*, 30(2): 3468-3472.
7. Imafidon K.E. and Amaechina F.C., (2010). Effects of aqueous seed extract of *Persea americana* Mill. (Avocado) on blood pressure and lipid profile in hypertensive rats. *Adv. Biol. Res.*, 4(2): 116-121.
8. Daramola J.O., Onanuga O.D., Abioja O.M., Adeleke M., Olowofeso O., Oke O.E., Adekunle E.O., Sorongbe T.A., Iyanda O.A., (2016). Effects of avocado seed extract in different trisextenders on sperm and oxidative stress indices of vitrified goat spermatozoa. *J. Agri. Sci.*, 61(4): 359-374.
9. Umeaku C.N., Chris-Umeaku C.I., Emmy-egbe I.O., Ukoha C.C., Uzor U.C., Agbo U.J., (2018). Proximate, phytochemical and antibacterial analysis of *Persea americana* obtained from Nigeria. *J. Dis. Med. Plants*, 4(3): 89-95.
10. Talabi J., Olukemi A.O., Ajayi O.O., Adegoke G.O., (2016). Nutritional and antinutritional compositions of processed Avocado (*Persea americana* Mill) seeds. *Asian J. Plant Sci. Res.*, 6(2): 6-12.
11. Imafidon E.K. and Okunrobo O.L., (2009). Biochemical evaluation of the tradomedicinal ues of seeds of *Persea americana* Mill., (family : Lauraceae). *World J. Med. Sci.*, 4(2): 143-146.
12. Sahar M.M.O. and Abeer A.A.E.S., (2007). Modified vaginal smear cytology for the determination of the rat estrous cycle phases, versus ordinary Papanicolaou technique, verifie by light and scanning electron microscopic examination of the endometrium. *Egypt. J. Histol.*, 30(2):397-408.
13. Nilsson U.A., Olsson L.I., Carlin G., Bylund-Fellenius A.C., (1989). Inhibition of lipid peroxidation by spin labels. *J. Biol. Chem.*, 264(19): 11131-11135.
14. Misra H.P.and Fridovich I., (1972). The generation of superoxide radical during the autoxidation of hemoglobin. *J. Biol. Chem.*, 247(21): 6960-6962.
15. Sinha A.K., (1972). Colorimetric assay of catalase. *Anal. Biochem.*, 47(2): 389-394.
16. Habbu P.V., Shastry R.A., Mahadevan K.M., Hanumanthachar J., Das S.K., (2008). Hepatoprotective and antioxidant effects of *Argyreia speciosa* in rats. *Afr. J. Tradit. Complement. Altern. Med.*, 5(2): 158-164.
17. Tchoffo H., Kana J.R., Ngoula F., Ngoumtsop V.H., Ngouozeu M.W.M., Tadondjou C.D'A., Folack T.L.V., (2019). Effects of ginger (*Zingiber officinale,* Roscoe) essential oil on growth and laying performances, serum metabolites, and egg yolk antioxidant and cholesterol status in laying japanese quail. *J. Vet. Med.*, Vol. 2019, Article ID 7857504, Pp. 8.
18. Gnanagurudasan E., Senthil K.S.K., Leena D.J.,

(2017). Comparative study on the estimation of estrous cycle in mice by visual and vaginal lavage method. *J. Clin. Diagnostic Res.*, 11(1): AC05-AC07.

19. Ngadjui E., Watcho P., Nguelefack T.B., Kamanyi A., (2013). Effects of *Ficus asperifolia* on normal rat estrus cyclicity. *Asian Pac. J. Trop. Biomed.*, 3(1): 53 -57.
20. Nayanatara A.K., Akshatha A., Sharannya K., Anwar A.S., Rejecsh E.P., Bhagyalak S., Sneha B.S., Rekhad K., Sheila R.P., (2012). Effect of *Cynodon dactylon* extract on estrous cycle and reproductive organs in female wistar rats. *Int. J. Anal., Pharmaceut. Biomed. Sci.*, 1(3): 10-15.
21. Ososki A.L. and Kennelly E.J., (2003). Phytoestrogens: a review of the present state of research. *Phytother. Rese.*, 17: 845-869.
22. Mineta T., Norikoshi K., Yamashita J., (2001). Induction of persistent estrus by early postnatal exposure to isoflavone in mice. *Environ Sci.*, 8, 515.
23. Awounfack C.F., Mvondo M.A., Zingue S., Ateba S.B., Djiogue S., Megnekou R., Ndinteh D.T., Njamen D., (2018). *Myrianthus arboreus* P. Beauv (Cecropiaceae) extracts accelerates sexual maturation, and increases fertility index and gestational rate in femaleWistar rats. *Medicines*, 5: Pp.73.
24. Krishnamoorthy P., Sivaranjani K., Rajeswari K., Kalaiselvan D., (2012). Effect of *Andrographis paniculata* Wall. Ex Nees root extract fractions on estrogen, FSH, LH, progesterone and ovary of female albino rats, *Rattus norvegicus*. *Indian J. Nat. Prod. Resour.*, 4(1): 42-47.
25. Retana-Márquez S., Hernández H., Flores J.A., Muñoz-Gutiérrez M., Duarte G., Vielma J., Fitz-Rodriguez G., Fernández I.G., Keller M., Delgadillo J.A., (2012). Effects of phytoestrogens on mammalian reproductive physiology. *Trop. Subtrop. Agroecosystems*, 15(1): S129-S145.
26. Gayrard V., (2007). Physiologie de la reproduction des mammifères. Polycopié. Unité de physiologie-physiopathologie. Ecole nationale vétérinaire de Toulouse, Pp. 198.
27. Nwaoguikpe R.N.and Braide W., (2011). The effect of aqueous extract of *Persea americana* (Avocado, Pear) on serum lipid and cholesterol levels in rabbits. *Afr. J. Pharmacy Pharmacol. Res.*, 1(2): 023 -029.
28. Grandjean D., Renard N., Dhote F., Ouabdesselam M., Aubert L., Bicard X., (2005). Modèle d'étude du stress oxydatif cellulaire - le chien de travail en environnement extrême. *Le Nouveau Praticien Vétérinaire*, 121: 37-40.
29. Zelena D., (2015). The janus face of stress on reproduction: From heath to disease. *Int. J. Endocrinol.*, Vol. 2015, Article ID 458129, Pp. 10.
30. Guiekep A.J.M., Kenfack A., Ngoula F., Vemo N.B., Nguemmeugne K.S., Pamo E.T., (2019). Attenuating effects of *Mangifera indica* leaves ethanolic extract against acetamiprid induced reproductive toxicity in male guinea pigs. *Vet. Res. Forum*, 10(3): 187-192.
31. Chongsi M.M.M., Ngoula F., Ngouateu K.O.B., Makona N.A.M., Kenfack A., Vemo N.B., Tchoffo H., Tchoumboue J., (2019). Oxidative effects of potassium dichromate on biochemical, hematological characteristics, and hormonal levels in rabbit doe (*Oryctolagus cuniculus*). *J. Vet. Sci.*, 6: Pp. 30.
32. Kath R.K. and Gupta R.K., (2006). Antioxidant activity of hydroalcoholic leaf extract of *Ocimum sanctum* in animal models of peptic ulcer. *Indian J. Physiol. Pharmacol.*, 50(4): 391-396.
33. Padilla-Camberos E., Martínez-Velázquez M., Flores-Fernández J.M., Villanueva-Rodríguez S., (2013). Acute toxicity and genotoxic activity of avocado seed extract (*Persea americana* Mill., c.v. *Hass*). *Sci. World J.*, Vol. 2013, Article ID 245828, Pp. 4.
34. Hennessey-Ramos L., Murillo-Arango W., Guayabo G.T., (2019). Evaluation of a colorant and oil extracted from avocado waste as functional components of a liquid soap formulation. *Rev. Fac. Nac. Agron. Medellín*, 72(2): 8855-8862.
35. Boyadzhieva S.S., Georgieva S.S., Angelov G., (2019). Optimization of the extraction of natural antioxidants from avocado seeds. *Bulg. Chem. Commun.*, 50: 80-84.